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(54) Title: RECEPTOR FOR ONCOSTATIN M AND LEUKEMIA INHIBITORY FACTOR

(57) Abstract

A receptor protein comprising a gp130 polypeptide linked to a single-chain leukemia inhibitory factor receptor (LIF-R) polypeptide is capable of binding both oncostatin M and leukemia inhibitory factor (LIF). The receptor protein binds LIF with greater affinity than does the single-chain LIF-R polypeptide alone. The receptor may be produced as a fusion protein in recombinant cells. The gp130 polypeptide binds oncostatin M, but with lower affinity than does the inventive receptor protein.

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TITLE

Receptor for Oncostatin M and Leukemia Inhibitory Factor

BACKGROUND OF THE INVENTION

Receptors that bind specific molecules (e.g., a hormone, drug, cytokine, or biochemical) have been identified on a multitude of cell types. Receptors are found on the cell surface or, in the case of soluble receptors, are released into the serum. Effort has been directed toward isolation and characterization of a number of receptors in order to study their physiological roles and to explore possible therapeutic uses. The binding of a particular target molecule by a soluble receptor administered to a patient may alleviate disorders mediated by the target molecule.

Certain receptors have been found to comprise two separate polypeptide chains associated in the form of a complex. Such two-chain receptors often bind the target molecule with greater affinity than that exhibited by one of the chains alone.

Leukemia inhibitory factor (LIF) is a polypeptide hormone that plays a central role in the regulation of diverse adult and embryonic systems. LIF acts on a variety of cell types and has multiple biological activities. The diversity in biological activity is reflected in the various synonyms of LIF, which include hepatocyte stimulating factor III (Baumann and Wong, *J. Immunol.* *143*:1163 [1989]); cholinergic nerve differentiation factor (Yamamori et al., *Science 246*: 1412 [1990]); melanoma-derived lipoprotein lipase inhibitor (Mori et al., *Biochem. Biophys. Res. Comm. 160*:1085 [1989]); human interleukin for DA cells (Moreau et al., *Nature 336*:690 [1988]); differentiation factor (Tomida et al., *J. Biol. Chem. 259*:10978 [1984]); differentiation inhibitory factor (Abe et al., *J. Biol. Chem. 264*; 8941 [1989]); differentiation inhibitory activity (Smith and Hooper, *Devel. Biol;. 121*:1 [1987]); and differentiation retarding factor (Koopman and Cotton, *Exp. Cell. Res. 154*:233 [1984].

The cloning of a leukemia inhibitory factor receptor (LIF-R) has been reported by Gearing et al. in *EMBO J. 10*:2839 (1991). This recombinant single-chain LIF-R polypeptide binds LIF, but with lower affinity than the naturally occurring LIF

receptors found on certain normal cells. A receptor that binds LIF with higher affinity than that exhibited by this cloned single chain LIF-R would be desirable for certain applications.

Oncostatin M is a secreted single-chain polypeptide cytokine that regulates the growth of certain tumor-derived and normal cell lines. Oncostatin M is produced by activated lymphoid cells. A number of cell types have been found to bind the oncostatin M protein. See, for example, Linsley et al., *J. Biol. Chem.*, 264: 4282 (1989). However, the isolation and characterization of an oncostatin M receptor have not been reported.

SUMMARY OF THE INVENTION

The present invention provides a receptor that has the property of binding both oncostatin M and leukemia inhibitory factor (LIF). The receptor comprises gp130 linked (preferably covalently) to leukemia inhibitory factor receptor (LIF-R). The gp130 polypeptide may be covalently linked to the LIF-R polypeptide by any suitable means, such as *via* a cross-linking reagent or a polypeptide linker. In one embodiment of the invention, the receptor is a fusion protein produced by recombinant DNA technology. Disorders mediated by either oncostatin M or LIF may be treated by administering a therapeutically effective amount of the inventive receptor to a patient afflicted with such a disorder.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph presenting the results of an LIF binding assay. Host cells transfected with vector(s) encoding gp130 or LIF-R were assayed for the ability to bind LIF, as described in example 1.

Figure 2 is a graph presenting the results of an oncostatin M binding assay. Host cells transfected with vector(s) encoding gp130 or LIF-R were assayed for the ability to bind oncostatin M, as described in example 2.

Figure 3 is a graph depicting low affinity binding of oncostatin M to host cells transfected with a gp130 encoding expression vector, as described in example 2.

Figure 4 schematically depicts a receptor of the present invention wherein Fc polypeptides derived from an antibody are used to link a gp130 fragment to an LIF-R fragment.

Figure 5 presents composite DNA and encoded amino acid sequences of a full length LIF-R, determined by comparing the sequences of cDNA and genomic clones. The signal peptidase cleavage site is marked with a vertical arrow. The transmembrane region is heavily underlined. Potential N-linked glycosylation sites are marked with

asterisks. Hallmark residues associated with the hematopoietin family of receptors are shown boxed. The horizontal arrow marks the point at which genomic sequence was used to derive the 3' coding region of LIF-R, since the cDNA clones employed in determining this sequence terminated with a stretch of A nucleotides at this point.

Figure 6 presents the DNA and deduced amino acid sequences of cloned gp130 cDNA as reported by Hibi et al. in *Cell* 63:1149 (1990). A predicted signal sequence is underlined. The thick underline indicates a presumed transmembrane region. The sets of asterisks identify possible N-glycosylation sites.

Figure 7 presents Scatchard analyses that demonstrate the interaction of a soluble gp130/Fc fusion protein with soluble LIF-R/Fc in binding LIF and oncostatin M, as described in example 7.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a receptor comprising gp130 covalently linked to leukemia inhibitory factor receptor (LIF-R). In another embodiment of the invention, the receptor comprises gp130 non-covalently complexed with LIF-R. The receptor is capable of binding oncostatin M, and also binds leukemia inhibitory factor (LIF). The receptor thus is useful for treating disorders mediated by either oncostatin M or LIF.

The gp130 may be covalently linked to the LIF-R by any suitable means, such as *via* a cross-linking reagent or a polypeptide linker. The gp130 and LIF-R proteins are covalently linked in a manner that does not interfere with the resulting receptor's ability to bind oncostatin M and LIF. In one embodiment of the invention, the receptor is a fusion protein produced by recombinant DNA technology.

Non-covalent bonding of gp130 to LIF-R may be achieved by any suitable means that does not interfere with the receptor's ability to bind oncostatin M and LIF. In one approach, a first compound is attached to LIF-R and a second compound that will non-covalently bond to the first compound is attached to gp130. Examples of such compounds are biotin and avidin. The receptor is thus formed through the non-covalent interactions of biotin with avidin. In one embodiment of the invention, LIF-R and gp130 are recombinant polypeptides, each purified from recombinant cells and then non-covalently bonded together to form the receptor. A host cell may be transformed with two different expression vectors such that both LIF-R and gp130 are produced by the recombinant host cell. LIF-R and gp130 (one or both of which are soluble fragments as described below) produced by such transformed host cells may associate to form a complex through non-covalent interactions.

"Leukemia inhibitory factor receptor" (LIF-R) refers to a protein (a cytokine receptor) that is present on the surface of various hematopoietic cells, including monocyte-macrophages and megakaryocytes, and on non-hematopoietic cells, including osteoblasts, placental trophoblasts, and liver parenchymal cells. LIF-R is capable of binding leukemia inhibitory factor (LIF) molecules and plays a role in transducing the signal provided by LIF to a cell. In the absence of any species designation, LIF-R refers generically to mammalian LIF-R, which includes, but is not limited to, human, murine, and bovine LIF-R.

The cloning of human and murine leukemia inhibitory factor receptors (LIF-R), each a single polypeptide chain, has been reported by Gearing et al. in *EMBO J.* *10*:2839 (1991), which is hereby incorporated by reference in its entirety. The DNA sequence of a human LIF-R cDNA clone and the amino acid sequence encoded thereby are shown in SEQ ID NO: 5 and SEQ ID NO: 6. This cloned human cDNA encodes an N-terminal fragment of human LIF-R that includes (in order from N-terminus to C-terminus) a 44-amino acid signal sequence (amino acids -44 to -1), the entire extracellular region, a transmembrane region (the first amino acid of which is amino acid number 790 of SEQ ID NO: 5) and a portion of the cytoplasmic domain. The C-terminus of the fragment includes amino acids encoded by a poly-A segment and by a linker employed in vector construction, as described in Gearing et al., *supra*. The term "transmembrane region" as used herein refers to a string of hydrophobic amino acids positioned between the extracellular domain and the cytoplasmic domain of the protein. A plasmid vector containing the above-described cloned human LIF-R cDNA is designated pHLIFR-65 and has been deposited in *E. coli* host cells with the American Type Culture Collection on December 11, 1990 (ATCC accession no. 68491). The DNA and amino acid sequences of a full length native human LIF-R (determined by comparing the sequences of cDNA and genomic clones) have been reported by Gearing et al. *supra* and are presented herein in Figure 5.

The LIF-R encoded by the cloned cDNA (SEQ ID NO: 6) contains the entire extracellular region of LIF-R (the domain believed to be responsible for the LIF-binding activity), and binds LIF, but with lower affinity than does a naturally occurring LIF receptor found on certain normal cells. Additionally, oncostatin M competes with LIF for binding to naturally occurring high affinity LIF receptors on certain cell types (Gearing et al., *New Biologist*, 4:61, 1992) but did not bind to the above-described cloned LIF-R expressed in COS cells.

In order to investigate the possible existance of a high affinity converting subunit for the cloned single polypeptide chain LIF-R, host cells were co-transfected with the LIF-R encoding plasmid pHLIFR-65 and with pools from a human placental

cDNA library (also contained in an expression vector). The co-transfected cells were assayed for the ability to bind radiolabeled oncostatin M.

A positive cDNA pool was subdivided and the procedure repeated to isolate a single cDNA clone designated B10G that conferred the ability to bind oncostatin M on cells co-transfected with B10G and the LIF-R encoding plasmid pHLIFR-65. The co-transfected cells also were found to bind LIF with higher affinity than cells transfected with pHLIFR-65 alone. Host cells transfected with B10G alone exhibited low affinity oncostatin M binding sites. The B10G cloned cDNA was sequenced and found to encode a protein that is known as gp130.

Thus, it has now been found that a receptor comprising both LIF-R and gp130 binds LIF with higher affinity than does the single-chain LIF-R polypeptide alone. The improved LIF binding of LIF-R in combination with gp130 is described in example 1 below and depicted in Figure 1.

Although LIF does not bind to either high- or low-affinity oncostatin M receptors, it has now been found that oncostatin M binds to the receptors of the present invention comprising LIF-R and gp130. Oncostatin M binding is described in example 2 below and depicted in Figure 2.

A protein known as gp130 has been purified from cellular sources that include placental tissue and a myeloma cell line U266. A number of additional cell types have been found to express gp130 mRNA, as reported by Hibi et al., in *Cell 63*:1149 (1990). gp130 has been reported to be involved in the formation of high affinity interleukin-6 binding sites and in IL-6 signal transduction (Hibi et al. *supra)*. The cloning and expression of cDNA encoding a full length gp130 protein has been reported by Hibi et al., *supra*, which is hereby incorporated by reference in its entirety. The DNA and deduced amino acid sequences reported by Hibi et al. for the gp130 cloned cDNA are presented herein in figure 6. The gp130 amino acid sequence may vary from that reported by Hibi et al., e.g., leucine may be substituted for valine at position 8 in the signal sequence (numbering is as shown in Figure 6). This amino acid substitution may be attributable to genetic polymorphism (allelic variation among individuals producing the protein), and results from the presence of C rather than G at nucleotide position 22.

As used herein, the term LIF-R includes variants and truncated forms of native LIF-R proteins that possess the desired LIF-binding or signal transducing activity. Likewise, the term gp130 as used herein includes variants and truncated forms of the native gp130 protein that retain the desired biological activity. For gp130, the desired biological activity includes binding of oncostatin M; conferring on the inventive receptor the ability to bind oncostatin M; and increasing the affinity of the inventive

receptor for LIF, compared to the LIF binding affinity of the single-chain LIF-R polypeptide alone. Specifically included are truncated, soluble or fusion forms of LIF-R and gp130, as described below. Variants produced by adding, substituting, or deleting amino acid(s) in the native sequence are discussed in more detail below.

One example of an LIF-R polypeptide that may be employed is that encoded by the cDNA clone designated pHLIF-R-65 (SEQ ID NO: 5), as described by Gearing et al., *supra* and in example 3 below. Alternatively, a fragment comprising amino acids 1 to 945 of SEQ ID NO:5 may be employed. Amino acid 945 is the last LIF-R-specific amino acid of the polypeptide encoded by clone pHLIF-R-65, before the poly-A nucleotide segment believed to result from oligo(dT) priming at an internal site in the mRNA during preparation of the hLIF-R cDNA. (See Gearing et al., *EMBO J., supra* at page 2840, column one.)

Other examples of LIF-R polypeptides that may be employed in the inventive receptors include those lacking all or part of the transmembrane region or the cytoplasmic domain of the protein. Suitable LIF-R polypeptides thus include those containing amino acids 1-x or, when the signal sequence is not desired, amino acids 45-x of the full length LIF-R sequence depicted in Figure 5, wherein x represents an integer from 833 to 1096. Amino acid number 833 is the last amino acid of the extracellular domain (i.e., before the start of the transmembrane region.) Polypeptides terminating in amino acid number 1096 lack the last C-terminal amino acid of the full length protein. The desirability of including the signal sequence depends on such factors as the position of LIF-R in a fusion protein, as discussed below, and the intended host cells when the receptor is to be produced *via* recombinant DNA technology. Note that the numbering of amino acids in Figure 5 (taken from Gearing et al., *supra*) differs from that of SEQ ID NO: 5 because the first amino acid of the signal sequence is designated amino acid number 1 in Figure 5 but is designated -44 in SEQ ID NO: 5. Other polypeptides may be chosen with regard to sequences that are conserved in the hematopoietin receptor family, (i.e., chosen to include the boxed sequence(s) shown in Figure 5.)

One example of a suitable gp130 polypeptide is that encoded by cDNA cloned into plasmid vector pDC303 to produce a plasmid designated B10G. The source of mRNA used in producing the cDNA was human placental tissue. Plasmid B10G in *E. coli* strain DH5α host cells was deposited with the American Type Culture Collection, Rockville, Maryland, on November 14, 1991, and assigned ATCC accession number 68827.

The DNA sequence of the gp130 cDNA contained in plasmid B10G and the amino acid sequence of the gp130 protein encoded by the cloned cDNA are presented in

SEQ ID NO: 1 and SEQ ID NO: 2. The protein comprises (in order from the N-terminus to the C-terminus) a 22-amino acid signal sequence, complete extracellular domain (amino acids 1-597), a transmembrane region (beginning with amino acid 598), and a partial cytoplasmic domain (amino acids 621-686). This truncated gp130 polypeptide differs from the equivalent portion of the Hibi et al. protein in that the eighth amino acid of the signal sequence is leucine rather than valine, as discussed above.

Another example of a suitable gp130 polypeptide comprises amino acids 1 to 496 of the SEQ ID NO: 1, which includes all of the cysteine residues found in the extracellular domain of the protein, and also contains a complete fibronectin domain. Additional examples of gp130 polypeptides are those comprising amino acids 1-298 or 98-298 of SEQ ID NO: 1.

Other gp130 polypeptides lacking all or part of the transmembrane region and/or cytoplasmic domain may be employed. Suitable gp130 polypeptides thus include those containing amino acids 1-x or, when the signal sequence is not desired, amino acids 23-x of the Figure 6 sequence, wherein x represents an integer from 619 to 917. The first amino acid of the transmembrane region is the alanine residue at position 620 in Figure 6. Polypeptides terminating at amino acid 917 lack the last C-terminal amino acid of the full length protein presented in Figure 6. Note that the numbering of amino acids in Figure 6 (taken from Hibi et al., *supra*) differs from that shown in SEQ ID NO: 1 and NO:2 because the first amino acid of the signal sequence is designated amino acid number 1 in Figure 6 but is designated -22 in SEQ ID NO: 1. Regions of the gp130 protein corresponding to domains that are conserved among certain receptors are discussed by Hibi et al, *supra*, at page 1150, column 2, and page 1151, column 1. Other truncated gp130 polypeptides chosen to include these conserved regions may be employed.

Preferred LIF-R and gp130 polypeptides are those which are soluble. In one embodiment of the present invention, the receptor comprises soluble LIF-R covalently attached to soluble gp130. "Soluble LIF-R" as used in the context of the present invention refers to polypeptides that are substantially similar in amino acid sequence to all or part of the extracellular region of a native LIF-R and that, due to the lack of a transmembrane region that would cause retention of the polypeptide on a cell membrane, are secreted upon expression. The soluble LIF-R polypeptides that may be employed retain the ability to bind LIF or, by competitively binding LIF, inhibit LIF signal transduction activity via cell surface bound LIF-R proteins. Soluble LIF-R may also include part of the transmembrane region or part of the cytoplasmic domain or other sequences, provided that the soluble LIF-R protein is capable of being secreted.

Likewise, the term "soluble gp130" as used herein refers to proteins that are substantially similar in amino acid sequence to all or part of the extracellular region of a native gp130 and are secreted upon expression but retain the desired biological activity. Soluble gp130 may include part of the transmembrane region, cytoplasmic domain, or other sequences, as long as the polypeptide is secreted.

Soluble LIF-R and soluble gp130 may be identified (and distinguished from their non-soluble membrane-bound counterparts) by separating intact cells which express the desired protein from the culture medium, e.g., by centrifugation, and assaying the medium (supernatant) for the presence of the desired protein. The culture medium may be assayed using procedures which are similar or identical to those described in the examples below. The presence of LIF-R or gp130 in the medium indicates that the protein was secreted from the cells and thus is a soluble form of the desired protein. Soluble LIF-R and soluble gp130 may be naturally-occurring forms of these proteins. Cloning of a naturally-occurring soluble murine LIF-R is reported in Gearing et al., *supra*. Alternatively, soluble fragments of LIF-R and gp130 proteins may be produced by recombinant DNA technology or otherwise isolated, as described below.

The use of soluble forms of LIF-R and gp130 is advantageous for certain applications. Purification of the proteins from recombinant host cells is facilitated, since the soluble proteins are secreted from the cells. Further, soluble proteins are generally more suitable for intravenous administration and may exert their therapeutic effect (binding LIF and oncostatin M) in the bloodstream.

Soluble LIF-R polypeptides include those comprising the signal sequence and entire extracellular domain (amino acids -44 to 789 of SEQ ID NO: 5) or lacking the signal sequence but containing the entire extracellular domain (amino acids 1 to 789 of SEQ ID NO: 5). Soluble gp130 polypeptides include those comprising the signal sequence and entire extracellular domain (amino acids -22 to 597 of SEQ ID NO: 1) or lacking the signal sequence but containing the entire extracellular domain (amino acids 1 to 597 of SEQ ID NO: 1). The preparation and use of these soluble polypeptides in receptors of the present invention is described in examples 3-5.

Other soluble LIF-Rs are truncated upstream of the transmembrane region, but preferably include that portion of the protein that contains the residues conserved among the members of the hematopoietin receptor family (shown boxed in Figure 5), i.e., amino acids 11-479 of SEQ ID NO:6. The N-terminus of such soluble LIF-Rs is any of amino acids 1-11 (or -44 when the native signal sequence is included), and the protein extends to a C-terminus selected from any of amino acids 479 through 789. Two such soluble proteins comprise amino acids -44 - 702, 1 - 702, -44 - 775, or 1 -

755 of SEQ ID NO:6. Constructs encoding these proteins may be prepared by techniques that involve cleaving the human LIF-R cDNA of the above-described clone pHLIFR-65 (ATCC 68491) with the restriction endonucleases *Asp718* and *XmnI* or with *Asp718* and *Bsp1286I*. *Asp 718* cleaves the vector upstream of the inserted LIF-R-encoding cDNA. *XmnI* cleaves within the codon for Asp at position 702 (generating blunt ends) and *Bsp1286I* cleaves just 3' of the codon for Val at position 775 of SEQ ID NO:5. If desired, an oligonucleotide may be ligated to the 3' end of the *Asp718/Bsp1286I* fragment to extend the LIF-R sequence, e.g., through amino acid number 789. An oligonucleotide also may be ligated to the 3' end of a LIF-R fragment to add the first two amino acids of the Fc polypeptide described in example 5, and a Bgl II site useful for attaching the rest of the Fc sequence downstream of the LIF-R sequence.

Additional soluble human LIF-Rs comprise amino acids 1-678 or 1-680 of SEQ ID NO:6. When the human and murine LIF-R amino acid sequences disclosed in Gearing et al., *EMBO J.*, *supra*, are aligned (with gaps introduced to maximize identity between the two sequences), amino acid 680 of the human sequence is aligned with the last amino acid of the murine protein, and amino acid 678 is the last amino acid of the human sequence that is identical to a corresponding amino acid in the murine sequence. Since the murine protein binds LIF, the murine LIF-R contains that portion of the protein required for LIF binding.

An additional example of a soluble gp130 polypeptide comprises amino acids -22 to 582 of SEQ ID NO:2. An expression vector encoding such a protein was constructed in example 7. Soluble LIF-R and gp130 polypeptides also include those from which fibronectin type III (FNIII) domains have been deleted. From one to all of the FNIII domains may be deleted, providing the advantage of reducing the size of the protein. Preparation of such LIF-R and gp130 proteins is described in example 8.

Truncated LIF-R and gp130, including soluble polypeptides, may be prepared by any of a number of conventional techniques. In the case of recombinant proteins, a DNA fragment encoding a desired fragment may be subcloned into an expression vector. Alternatively, a desired DNA sequence may be chemically synthesized using known techniques. DNA fragments also may be produced by restriction endonuclease digestion of a full length cloned DNA sequence, and isolated by electrophoresis on agarose gels. Linkers containing restriction endonuclease cleavage site(s) may be employed to insert the desired DNA fragment into an expression vector, or the fragment may be digested at cleavage sites naturally present therein. Alternatively, proteins may be fragmented using proteolytic enzymes, for example, and the desired truncated polypeptide isolated from the digestion mixture using reversed phase HPLC.

The well known polymerase chain reaction procedure also may be employed to isolate a DNA sequence encoding a desired protein fragment. This technique is illustrated in examples 3-5 below.

In another approach, enzymatic treatment (e.g., using Bal 31 exonuclease) may be employed to delete terminal nucleotides from a DNA fragment to obtain a fragment having a particular desired terminus. Among the commercially available linkers are those that can be ligated to the blunt ends produced by Bal 31 digestion, and which contain restriction endonuclease cleavage site(s). Alternatively, oligonucleotides that reconstruct the N- or C- terminus of a DNA fragment to a desired point may be synthesized. The oligonucleotide may contain a restriction endonuclease cleavage site upstream of the desired coding sequence and position an initiation codon (ATG) at the N-terminus of the coding sequence.

The gp130 polypeptide is attached to the LIF-R polypeptide through a covalent or non-covalent linkage. Covalent attachment is preferred for certain applications, e.g. *in vivo* use, in view of the enhanced stability generally conferred by covalent, as opposed to non-covalent, bonds. In constructing the receptor of the present invention, covalent linkage may be accomplished *via* cross-linking reagents, polypeptide linkers, or any other suitable technique.

Numerous reagents useful for cross-linking one protein molecule to another are known. Heterobifunctional and homobifunctional linkers are available for this purpose from Pierce Chemical Company, Rockford, Illinois, for example. Such linkers contain two functional groups (e.g., esters and/or maleimides) that will react with certain functional groups on amino acid side chains, thus linking one polypeptide to another. The reagent and reaction conditions should be chosen such that the cross-linking does not interfere with binding of oncostatin M and LIF to the receptor.

One type of polypeptide linker that may be employed in the present invention separates gp130 and LIF-R domains by a distance sufficient to ensure that each domain properly folds into the secondary and tertiary structures necessary for the desired biological activity. The linker also should allow the extracellular domains of gp130 and LIF-R to assume the proper spatial orientation to form the binding site for oncostatin M and LIF. Suitable polypeptide linkers preferably (1) will adopt a flexible extended conformation, (2) will not exhibit a propensity for developing an ordered secondary structure which could interact with the functional gp130 and LIF-R domains, and (3) will have minimal hydrophobic or charged character which could promote interaction with the functional protein domains. Typical surface amino acids in flexible protein regions include Gly, Asn and Ser. Virtually any permutation of amino acid sequences containing Gly, Asn and Ser would be expected to satisfy the above criteria for a

peptide linker sequence. Other near neutral amino acids, such as Thr and Ala, may also be used in the linker sequence. Examples of such polypeptide linkers are presented below.

Another type of polypeptide linker that may be employed comprises the Fc region of an antibody. An Fc polypeptide is attached to the C-terminus of LIF-R or of the LIF-R fragment. A separate Fc polypeptide is attached to the C-terminus of gp130 or of the gp130 fragment. The two resulting polypeptide chains may be combined in a buffered solution, whereupon disulfide bonds form between the two Fc polypeptides (e.g., in the so-called hinge region, where interchain disulfide bonds are normally present in antibody molecules). Preferably, a host cell is transformed with DNA encoding both polypeptides such that the two polypeptides are co-expressed and interchain disulfide bonds form in the host cell. LIF-R is thus covalently linked to gp130 via the disulfide bonds in the linker portion of the receptor. Procedures for isolating the Fc region of an antibody are well-known and include proteolytic digestion with papain. Alternatively, an Fc polypeptide may be produced by recombinant cells or chemically synthesized. Also useful are N-terminal fragments of an antibody Fc region that contain the cysteine residues involved in disulfide bond formation at the hinge region. One example of a receptor containing an Fc polypeptide linker is illustrated in example 5 below. The receptor is depicted in Figure 4. The number and position of disulfide bonds may vary from those shown in Figure 4.

Additional examples of LIF-R/Fc and gp130/Fc fusion proteins useful in preparing receptors of the present invention are described in examples 7 and 8. Advantageously, host cells are co-transfected with two different expression vectors, one encoding soluble LIF-R/Fc and the other encoding soluble gp130/Fc. The heterodimer is believed to form intracellularly or during secretion.

Homodimers comprising two LIF-R/Fc polypeptides or two gp130/Fc polypeptides linked via disulfide bonds are also produced by certain of the transfected host cells disclosed herein. The LIF-R/Fc homodimers are useful for binding LIF and the gp130/Fc homodimers find use in binding oncostatin M. The homodimers may be separated from each other and from the heterodimer by virtue of differences in size (e.g., by gel electrophoresis). The heterodimer also may be purified by sequential immunoaffinity chromatography (described below).

In an alternative embodiment, a first fusion polypeptide comprising gp130 (or fragment thereof) upstream of an antibody light chain (or a fragment thereof) is prepared. A second second fusion polypeptide comprises LIF-R upstream of an antibody heavy chain (or a heavy chain fragment, the N-terminus of which extends at least through the C_H1 region. Disulfide bond(s) form between the gp130-light chain

fusion polypeptide and the LIF-R-heavy chain fusion polypeptide, thus producing a receptor of the present invention comprising a polypeptide linker. If desired, a third fusion (an LIF-R-antibody light chain fusion polypeptide) is prepared and combined with (disulfide bonded to) a fourth fusion comprising gp130 fused to an antibody heavy chain. When the two disulfide bonded molecules are combined, additional disulfide bonds form between the two Fc regions. The resulting receptor of the present invention comprising the four fusion polypeptides resembles an antibody in structure and displays the oncostatin M/LIF binding site bivalently.

A polypeptide linker may be attached to gp130 and to LIF-R by any of the conventional procedures used to attach one polypeptide to another. The cross-linking reagents available from Pierce Chemical Company as described above are among those that may be employed. Amino acids having side chains reactive with such reagents may be included in the polypeptide linker, e.g., at the termini thereof.

The gp130 and LIF-R polypeptides may be separately purified from cellular sources, and then linked together. Alternatively, the receptor of the present invention may be produced using recombinant DNA technology. The gp130 and LIF-R polypeptides may be produced separately and purified from transformed host cells for subsequent covalent linkage. In one embodiment of the present invention, a host cell is transformed/transfected with foreign DNA that encodes gp130 and LIF-R as separate polypeptides. The two polypeptides may be encoded by the same expression vector with start and stop codons for each of the two genes, or the recombinant cells may be co-transfected with two separate expression vectors. In another embodiment, the receptor is produced as a fusion protein in recombinant cells.

In one embodiment of the present invention, the receptor protein is a recombinant fusion protein of the formula:

$$R_1\text{-}L\text{-}R_2 \text{ or } R_2\text{-}L\text{-}R_1$$

wherein R_1 represents gp130 or a gp130 fragment; R_2 represents LIF-R or an LIF-R fragment; and L represents a polypeptide linker.

The fusion proteins of the present invention include constructs in which the C-terminal portion of gp130 is fused to the linker which is fused to the N-terminal portion of LIF-R, and also constructs in which the C-terminal portion of LIF-R is fused to the linker which is fused to the N-terminal portion of gp130. gp130 is covalently linked to LIF-R in such a manner as to produce a single protein which retains the desired biological activities of gp130 and LIF-R. The components of the fusion protein are listed in their order of occurrence (i.e., the N-terminal polypeptide is listed first, followed by the linker and then the C-terminal polypeptide).

A DNA sequence encoding a fusion protein is constructed using recombinant DNA techniques to insert separate DNA fragments encoding gp130 and LIF-R into an appropriate expression vector. The 3' end of a DNA fragment encoding gp130 is ligated (*via* the linker) to the 5' end of the DNA fragment encoding LIF-R with the reading frames of the sequences in phase to permit translation of the mRNA into a single biologically active fusion protein. Alternatively, the 3' end of a DNA fragment encoding LIF-R may be ligated (*via* the linker) to the 5' end of the DNA fragment encoding gp130, with the reading frames of the sequences in phase to permit translation of the mRNA into a single biologically active fusion protein. A DNA sequence encoding an N-terminal signal sequence may be retained on the DNA sequence encoding the N-terminal polypeptide, while stop codons, which would prevent read-through to the second (C-terminal) DNA sequence, are eliminated. Conversely, a stop codon required to end translation is retained on the second DNA sequence. DNA encoding a signal sequence is preferably removed from the DNA sequence encoding the C-terminal polypeptide.

Suitable polypeptide linkers comprise a chain of amino acids, preferably from 20 to 100 amino acids in length and most preferably from 30 to 60 amino acids in length. As discussed above, the linker advantageously comprises amino acids selected from the group consisting of glycine, asparagine, serine, threonine, and alanine. Examples of suitable polypeptide linkers include, but are not limited to, $(Gly_4Ser)_n$, wherein n is 4-12, preferably 8, and $(Gly_4SerGly_5Ser)_2$.

A DNA sequence encoding a desired polypeptide linker may be inserted between, and in the same reading frame as, the DNA sequences encoding gp130 and LIF-R using any suitable conventional technique. For example, a chemically synthesized oligonucleotide encoding the linker and containing appropriate restriction endonuclease cleavage sites may be ligated between the sequences encoding gp130 and LIF-R.

Alternatively, a chemically synthesized DNA sequence may contain a sequence complementary to the 3' terminus (without the stop codon) of either gp130 or LIF-R, followed by a linker-encoding sequence which is followed by a sequence complementary to the 5' terminus of the other of gp130 and LIF-R. Oligonucleotide directed mutagenesis is then employed to insert the linker-encoding sequence into a vector containing a direct fusion of gp130 and LIF-R.

The present invention provides an isolated DNA sequence encoding the above-described fusion protein comprising gp130, LIF-R, and a polypeptide linker, and also provides recombinant expression vectors containing the isolated DNA sequence. "Expression vector" refers to a replicable DNA construct used to express DNA which

encodes the desired protein and which includes a transcriptional unit comprising an assembly of (1) genetic element(s) having a regulatory role in gene expression, for example, promoters, operators, or enhancers, operatively linked to (2) a DNA sequence encoding a desired protein (in this case, a receptor of the present invention) which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. The choice of promoter and other regulatory elements generally varies according to the intended host cell.

Proteins to be produced in yeast expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by the yeast host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue. This residue optionally may be subsequently cleaved from the expressed recombinant protein to provide a final product.

In the expression vectors, regulatory elements controlling transcription or translation are generally derived from mammalian, microbial, viral or insect genes. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated. Vectors derived from retroviruses also may be employed.

DNA regions are operably linked when they are functionally related to each other. For example, DNA encoding a signal peptide (secretory leader) is operably linked to DNA for a polypeptide if the polypeptide is expressed as a precursor that is secreted through the host cell membrane; a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, "operably linked" means contiguous and, in the case of secretory leaders, contiguous and in reading frame.

Transformed host cells are cells which have been transformed or transfected with foreign DNA using recombinant DNA techniques. In the context of the present invention, the foreign DNA includes a sequence encoding the inventive receptor. Host cells may be transformed for purposes of cloning or amplifying the foreign DNA, or may be transformed with an expression vector for production of the receptor protein. Suitable host cells for expression of the receptor include prokaryotes, yeast or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or bacilli. Prokaryotic expression vectors generally comprise one or more phenotypic selectable markers, for example a gene encoding proteins conferring antibiotic resistance or supplying an autotrophic requirement, and an origin of replication recognized by the host to ensure

amplification within the host. Examples of suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice. Higher eukaryotic cells include established cell lines of mammalian origin. Cell-free translation systems could also be employed to produce fusion protein using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (*Cloning Vectors: A Laboratory Manual*, Elsevier, New York, 1985), the relevant disclosure of which is hereby incorporated by reference.

Useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well-known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species (Bolivar et al., *Gene* 2:95, 1977). pBR322 contains genes for ampicillin and tetracycline resistance and this provides simple means for identifying transformed cells.

Promoters commonly used in recombinant microbial expression vectors include the b-lactamase (penicillinase) and lactose promoter system (Chang et al., *Nature* *275*:615, 1978; and Goeddel et al., *Nature 281*:544, 1979), the tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res.* 8:4057, 1980; and EPA 36,776) and tac promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful bacterial expression system employs the phage λ P_L promoter and cI857ts thermoinducible repressor. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λ P_L promoter include plasmid pHUB2, resident in *E. coli* strain JMB9 (ATCC 37092) and pPLc28, resident in E. coli RR1 (ATCC 53082).

The recombinant receptor protein may also be expressed in yeast hosts, preferably from *Saccharomyces* species, such as *S. cerevisiae*. Yeast of other genera such as *Pichia* or *Kluyveromyces* may also be employed. Yeast vectors will generally contain an origin of replication from the 2μm yeast plasmid or an autonomously replicating sequence (ARS), a promoter, DNA encoding the receptor fusion protein, sequences for polyadenylation and transcription termination and a selection gene. Preferably, yeast vectors will include an origin of replication and selectable markers

permitting transformation of both yeast and *E. coli*, e.g., the ampicillin resistance gene of *E. coli* and the *S. cerevisiae* trp1 gene, which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, and a promoter derived from a highly expressed yeast gene to induce transcription of a structural sequence downstream. The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable promoter sequences in yeast vectors include the promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149, 1968; and Holland et al., *Biochem.* 17:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman et al., EPA 73,657.

Preferred yeast vectors can be assembled using DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) and yeast DNA sequences including a glucose-repressible ADH2 promoter and α-factor secretion leader. The ADH2 promoter has been described by Russell et al. (*J. Biol. Chem.* 258:2674, 1982) and Beier et al., (*Nature* 300:724, 1982). The yeast α-factor leader, which directs secretion of heterologous proteins, can be inserted between the promoter and the structural gene to be expressed. See, e.g., Kurjan et al., *Cell* 30:922, 1982; and Bitter et al., *Proc. Natl. Acad. Sci. USA* 81:5330, 1984. The leader sequence may be modified to contain, near its 3' end, one or more useful restriction sites to facilitate fusion of the leader sequence to foreign genes.

Suitable yeast transformation protocols are known to those of skill in the art. An exemplary technique is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929, (1978), selecting for Trp$^+$ transformants in a selective medium consisting of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 μg/ml adenine and 20 μg/ml uracil.

Host strains transformed by vectors comprising the ADH2 promoter may be grown for expression in a rich medium consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 μg/ml adenine and 80 μg/ml uracil. Derepression of the ADH2 promoter occurs upon exhaustion of medium glucose. Crude yeast supernatants are harvested by filtration and held at 4°C prior to further purification.

Various mammalian or insect cell culture systems can be employed to express recombinant protein. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman (*Cell* 23:175, 1981), and other cell lines capable of expressing an appropriate vector including, for examples, L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines. Mammalian expression vectors may comprise non-transcribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a poly-adenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells may be provided by viral sources. For example, commonly used promoters and enhancers are derived from Polyoma, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. The early and late promoters are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin or replication (Fiers et al., *Nature* 273:113, 1978). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the Hind III site toward the *BglI* site located in the viral origin of replication is included. Exemplary vectors can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol.* 3:280, 1983).

A useful system for stable high level expression of mammalian receptor cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986).

Particularly preferred vectors for expression of the inventive receptor as a fusion protein are described in the examples below. The foregoing discussion is, of course, applicable to the production of recombinant fusion proteins comprising a fragment of gp130 and/or a fragment of LIF-R. Suitable fragments are discussed above, and DNA sequences encoding such fragments may be inserted into the above-described expression vectors.

The present invention provides a process for preparing the recombinant receptor of the present invention, comprising culturing a host cell transformed with an expression vector comprising a DNA sequence that encodes said receptor under

conditions that promote expression. The receptor is then purified from culture media or cell extracts.

For example, supernatants from systems which secrete recombinant protein into the culture medium can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. For example, a suitable affinity matrix can comprise LIF or OSM. An LIF affinity matrix may be prepared by coupling recombinant human LIF to cyanogen bromide-activated Sepharose (Pharmacia) or Hydrazide Affigel (Biorad), according to manufacturer's recommendations. Sequential immunopurification using antibodies bound to a suitable support is preferred. Proteins binding to an antibody specific for LIF-R are recovered and contacted with antibody specific for gp130 on an insoluble support. Proteins immunoreactive with both antibodies may thus be identified and isolated. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. One or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a fusion protein composition.

Some or all of the foregoing purification steps, in various combinations, can be employed to provide an essentially homogeneous recombinant protein. Recombinant cell culture enables the production of the fusion protein free of those contaminating proteins which may be normally associated with gp130 or LIF-R as they are found in nature in their respective species of origin, e.g., in cells, cell exudates or body fluids.

The foregoing purification procedures are among those that may be employed to purify non-recombinant receptors of the present invention as well. When linking procedures that may produce homodimers (gp130-linker-gp130 and LIF-R-linker-LIF-R) are employed, purification procedures that separate the desired heterodimer from such homodimers are employed. An example of such a procedure is sequential immunopurification as discussed above.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification

steps. Microbial cells employed in expression of recombinant fusion proteins can disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Fermentation of yeast which express fusion proteins as a secreted protein greatly simplifies purification. Secreted recombinant protein resulting from a large-scale fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog. 296*:171, 1984), involving two sequential, reversed-phase HPLC steps for purification of a recombinant protein on a preparative HPLC column.

The present invention also provides a pharmaceutical composition comprising a receptor protein of the present invention with a physiologically acceptable carrier or diluent. Such carriers and diluents will be nontoxic to recipients at the dosages and concentrations employed. Such compositions may, for example, comprise the receptor protein in a buffered solution, to which may be added antioxidants such as ascorbic acid, low molecular weight (less than about ten residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients. The receptor of the present invention may be administered by any suitable method in a manner appropriate to the indication, such as intravenous injection, continuous infusion, sustained release from implants, etc.

The DNA and/or amino acid sequences of gp130 and LIF-R may vary from those presented in SEQ ID NO: 1 and SEQ ID NO: 5. Due to the known degeneracy of the genetic code, there can be considerable variation in nucleotide sequences encoding the same amino acid sequence. The DNA sequences capable of hybridizing to the native DNA sequence of SEQ ID NO: 1 or SEQ ID NO: 5 under moderately stringent conditions (50°C, 2 X SSC), and which encode a biologically active gp130 or LIF-R polypeptide, are also considered to be gp130-encoding or LIF-R-encoding DNA sequences, respectively, in the context of the present invention. Further, certain mutations in a nucleotide sequence which encodes LIF-R or gp130 will not be expressed in the final protein product. For example, nucleotide substitutions may be made to enhance expression, primarily to avoid secondary structure loops in the transcribed mRNA (see EPA 75,444A, incorporated herein by reference). Other alterations of the nucleotide sequence may be made to provide codons that are more readily translated by the selected host, e.g., the well-known *E. coli* preference codons for *E. coli* expression.

The amino acid sequence of native gp130 or LIF-R may be varied by substituting, deleting, adding, or inserting one or more amino acids to produce a gp130 or LIF-R variant. Variants that possess the desired biological activity of the native

gp130 and LIF-R proteins may be employed in the receptor of the present invention. Assays by which the biological activity of variant proteins may be analyzed are described in the examples below.

Alterations to the native amino acid sequence may be accomplished by any of a number of known techniques. For example, mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (*Gene 42*:133, 1986); Bauer et al. (*Gene 37*:73, 1985); Craig (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); U.S. Patent No. 4,518,584, and U.S. Patent No. 4,737,462, which are incorporated by reference herein.

Bioequivalent variants of LIF-R and gp130 may be constructed by, for example, making various substitutions of amino acid residues or deleting terminal or internal amino acids not needed for biological activity. In one embodiment of the invention, the variant amino acid sequence is at least 80% identical, preferably at least 90% identical, to the native sequence. Percent similarity may be determined, for example, by comparing sequence information using the GAP computer program, version 6.0, available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilizes the alignment method of Needleman and Wunsch (J. Mol. Biol. 48:443, 1970), as revised by Smith and Waterman (Adv. Appl. Math. 2:482, 1981). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res. 14*:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

Generally, substitutions should be made conservatively; i.e., the most preferred substitute amino acids are those having physiochemical characteristics resembling those of the residue to be replaced. Examples of conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known. Moreover, particular amino acid differences between human, murine and other mammalian LIF-Rs is suggestive of additional conservative substitutions that may be made without altering the essential biological characteristics of LIF-R.

Cysteine residues can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. Adjacent dibasic amino acid residues may be modified to enhance expression in yeast systems in which KEX2 protease activity is present.

EP212,914 discloses the use of site-specific mutagenesis to inactivate KEX2 protease processing sites in a protein. KEX2 protease processing sites are inactivated by deleting, adding or substituting residues to alter Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these adjacent basic residues. Lys-Lys pairings are considerably less susceptible to KEX2 cleavage, and conversion of Arg-Lys or Lys-Arg to Lys-Lys represents a conservative and preferred approach to inactivating KEX2 sites.

Hydrophilic amino acids may be substituted for hydrophobic amino acids in the transmembrane region and/or intracellular domain of gp130 and LIF-R to enhance water solubility of the proteins. Addition of amino acids to the native sequence may result from translation of in-frame codons present in linkers used in constructing cloning or expression vectors. The LIF-R encoded by clone pHLIF-R-65 contains such linker-encoded amino acids at the C-terminus, as described by Gearing et al., *supra*.

The present invention also includes proteins with or without associated native-pattern glycosylation. Expression of DNAs encoding the fusion proteins in bacteria such as *E. coli* provides non-glycosylated molecules. Functional mutant analogs having inactivated N-glycosylation sites can be produced by oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast expression systems. N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn-A_1-Z, where A1 is any amino acid except Pro, and Z is Ser or Thr. In this sequence, asparagine provides a side chain amino group for covalent

attachment of carbohydrate. Such a site can be eliminated by substituting another amino acid for Asn or for residue Z, deleting Asn or Z, or inserting a non-Z amino acid between A_1 and Z, or an amino acid other than Asn between Asn and A_1. Known procedures for inactivating N-glycosylation sites in proteins include those described in U.S. Patent 5,071,972 and EP 276,846.

Variants of the receptor proteins of the present invention also include various structural forms of the primary protein which retain biological activity. Due to the presence of ionizable amino and carboxyl groups, for example, a receptor protein may be in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction.

The primary amino acid structure also may be modified by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives are prepared by linking particular functional groups to amino acid side chains or at the N- or C- termini. Other derivatives of the receptor protein within the scope of this invention include covalent or aggregative conjugates of the receptor protein with other proteins or polypeptides, such as by synthesis in recombinant culture as N- or C- terminal fusions. For example, the conjugated polypeptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast a-factor leader). Peptides may also be added to facilitate purification or identification of the fusion protein (e.g., poly-His). The amino acid sequence of the fusion protein can also be linked to the peptide Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (DYKDDDDK) (Hopp et al., *Bio/Technology* 6:1204, 1988) The latter sequence is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. This sequence is also specifically cleaved by bovine mucosal enterokinase at the residue immediately following the Asp-Lys pairing. Receptor proteins capped with this peptide may also be resistant to intracellular degradation in *E. coli*.

The receptors of the present invention are primarily useful as oncostatin M binding reagents, and may be administered *in vivo* to inhibit a biological activity of oncostatin M (including signal transduction). The inventive receptors also have use as LIF binding reagents.

Disorders mediated by either oncostatin M or LIF may be treated by administering a therapeutically effective amount of the receptor of the present invention to a human or mammalian patient afflicted with such a disorder. A disorder is said to

be mediated by oncostatin M or LIF when biologically active oncostatin M or LIF causes (directly or indirectly) or exacerbates the disorder. Soluble receptor proteins can be used to competitively bind to LIF and oncostatin M, thereby inhibiting binding of LIF and oncostatin M to cell surface receptors.

As discussed in example 2, gp130 has now been found to bind oncostatin M, although with lower affinity than the inventive receptors comprising both gp130 and LIF-R. gp130 may be administered to treat conditions mediated by oncostatin M, although a gp130/LIF-R receptor of the present invention would be preferred for such a purpose.

Oncostatin M has been reported to stimulate hematopoiesis, stimulate epithelial cell proliferation, increase plasmin activity (thereby inducing fibrinolysis), inhibit angiogenesis and supress expression of major histocompatibility complex antigens on endothelial cells. See PCT application WO 9109057 and European patent application no. 422,186. When these or other biological effects of oncostatin M are undesirable, a receptor of the present invention may be administered to bind oncostatin M.

Oncostatin M is believed to stimulate production of the cytokine interleukin-6 (IL-6), as reported by Brown et al., *J. Immunol. 147*:2175 (1991). Oncostatin M therefore indirectly mediates disorders associated with the presence of IL-6. IL-6 has been reported to be involved in the pathogenesis of AIDS-associated Kaposi's sarcoma (deWit et al., *J. Intern. Med. [England] 229*:539 [1991]). Binding of oncostatin M by a receptor of the present invention thus may be useful in treating Kaposi's sarcoma. Alternatively, but less preferably, gp130 may be administered to treat Kaposi's sarcoma.

Among the disorders mediated by LIF are lipoprotein metabolism defects such as atherosclerosis and obesity, as well as disorders of bone and calcium metabolism or disorders associated with LIF overproduction that affect hepatocytes, neurons, or leukocytes. The regulation of embryonic and hematopoietic stem cells by LIF may also be manipulated with the receptor. A soluble form of the receptor may also be used to treat leukemic cells which respond to LIF by proliferating. LIF also may play a role in inducing cachexia in cancer or AIDS patients. The receptor, or antibodies thereto, may also be useful as a diagnostic reagent to detect diseases characterized by the presence of abnormal LIF-R.

Oncostatin M and LIF are different proteins, but share certain structural and biological properties. If inhibition of a biological activity shared by oncostatin M and LIF is desired, the receptor of the present invention offers the benefit of binding both of these proteins exhibiting the particular biological activity. A receptor binding only one

of the proteins would leave the other protein active and continuing to mediate the disorder.

Receptor proteins or derivatives thereof may also be used as reagents in receptor-based immunoassays, reagents in assays for oncostatin M or LIF, or as binding agents for affinity purification of oncostatin M or LIF. The receptor proteins of the present invention may be used as immunogens in conventional procedures for production of polyclonal or monoclonal antibodies. Such antibodies may be employed on immunoaffinity columns for purification of the receptor, or as components of diagnostic or research assays. Derivatives may also be obtained by attaching additional polypeptide(s), e.g., by using a cross-linking agent, such as N-maleimidobenzoyl succinimide ester that reacts with cysteine and lysine residues. Receptor proteins may also be covalently bound through reactive side groups to various insoluble substrates, such as cyanogen bromide-activated, bisoxirane-activated, carbonyldiimidazole-activated or tosyl-activated agarose structures, or by adsorbing to polyolefin surfaces (with or without glutaraldehyde cross-linking).

The following examples are provided to illustrate certain embodiments of the invention, and are not to be construed as limiting the scope of the invention.

EXAMPLES

Example 1

Assay to Detect Binding of LIF

Recombinant human LIF is expressed in yeast and purified to homogeneity essentially as described by Hopp, et al., *Bio/Technology* 6:1204 (1988). The purified protein is radiolabeled using a commercially available enzymobead radioiodination reagent (BioRad). In this procedure 10 µg LIF in 50 µl 0.2 M sodium phosphate, pH 7.2, are combined with 50µl enzymobead reagent, 2 mCi of sodium iodide in 20 µl of 0.05 M sodium phosphate pH 7.0 and 10 µl of 2.5% ß-D-glucose. After 10 minutes at 25°C, sodium azide (20 µl of 50 mM) and sodium metabisulfite (10 µl of 5 mg/ml) are added and incubation is continued for 5 min. at 25°C. The reaction mixture is fractionated by gel filtration on a 2 ml bed volume of Sephadex® G-25 (Sigma) equilibrated in Roswell Park Memorial Institute (RPMI) 1640 medium containing 2.5% (w/v) bovine serum albumin (BSA), 0.2% (w/v) sodium azide and 20 mM Hepes pH 7.4 (binding medium). The final pool of ^{125}I-LIF is diluted to a working stock solution of 3 x 10^{-8} M in binding medium and stored for up to one month at 4°C

without detectable loss of receptor binding activity. The specific activity is routinely in the range of 6-8 x 10^{15} cpm/mmole LIF.

The radiolabeled LIF may be employed in any of a number of conventional assay procedures to determine whether a given protein or cell binds LIF. Examples of such assays are those that detect binding of the radiolabeled LIF to cells expressing an LIF-binding protein on the cell surface. The radiolabeled LIF also may be employed in assays for the presence of LIF-binding proteins in cell culture medium (e.g. LIF-binding proteins secreted by recombinant cells). Proteins in cell extracts (e.g. from recombinant cells) also may be assayed for the ability to bind the radiolabeled LIF.

In one assay procedure, cells transformed/transfected with an expression system encoding a protein to be tested for ability to bind LIF are plated at a density of 2 x 10^5 cells/well in either 6 well dishes (Falcon) or single well chambered slides (Lab-Tek). Both dishes and slides are pretreated with 1 ml human fibronectin (10 ug/ml in PBS) for 30 minutes followed by 1 wash with PBS. After 48 to 72 hours, cells are assayed for expression of LIF binding proteins by binding radioiodinated LIF using the following slide autoradiography technique. Transfected cells are washed once with binding medium (RPMI media 1640 containing 25 mg/ml bovine serum albumin (BSA), 2 mg/ml sodium azide, 20 mM HEPES, pH 7.2, and 50 mg/ml nonfat dry milk (NFDM) and incubated for 2 hours at 4°C with 1 ml binding medium + NFDM containing 1.25 x 10^{-9} M ^{125}I-LIF. After incubation, cells in the chambered slides are washed three times with binding buffer + NFDM, followed by 2 washes with PBS, pH 7.3, to remove unbound ^{125}I-LIF. The cells are fixed by incubating for 30 minutes at room temperature in 10% gluteraldehyde in PBS, pH 7.3, washed twice in PBS, and air dried. The slides are dipped in Kodak NTB-2 photographic emulsion (5x dilution in water) and exposed in the dark for 12 hours to 7 days at 4°C in a light proof box. The slides are then developed for approximately 5 minutes in Kodak D19 developer (40 g/500 ml water), rinsed in water and fixed in Agfa G433C fixer. The slides are individually examined with a microscope at 25-40x magnification and positive cells that bind LIF are identified by the presence of autoradiographic silver grains against a light background.

Cells in the 6 well plates are washed once with binding buffer + NFDM followed by 3 washings with PBS, pH 7.3, to remove unbound ^{125}I-LIF. The cells are then trypsinized to remove them from the plate and bound ^{125}I-LIF is counted on a gamma counter.

The cells in transfectant pool(s) testing positive are subdivided into smaller pools and the screening process is repeated (with further subdividing of the pools as necessary) until an individual clone expressing LIF-binding protein is isolated. Non-

specific binding of ^{125}I-LIF may be measured in the presence of 200-fold or greater excess of unlabeled LIF. As a control, the same host cells transfected with a vector lacking LIF-R-encoding sequences should be assayed to determine whether background endogenous LIF receptors are present on the host cells.

In another assay procedure, cells producing a soluble LIF-binding protein that is released from the cells into the culture medium may be identified. Cells are collected by centrifugation from a culture broth. The supernatant (culture medium) is concentrated 10-fold, and 1 µl aliquots are spotted onto nitrocellulose filters and allowed to air dry. Additional binding sites are blocked by overnight incubation at 4°C in the above-described binding medium containing 3% non-fat dry milk (BMNFDM). Filters are incubated for 2h at 4°C in BMNFDM containing 1 nM ^{125}I-LIF in the presence or absence of 200 nM unlabeled LIF, then washed (3 x 5 min) in PBS. Filters are exposed to photographic film for 48 hr at room temperature.

The results of one LIF binding assay conducted according to the following procedure are shown in Figure 1. Host cells transfected with vector(s) encoding LIF-R or gp130 as described below were assayed for the ability to bind LIF. The host cells were the monkey kidney cell line designated COS-7, described by Glutzman, *Cell* *23*:175 (1981). In separate transfections, COS-7 cells were transfected with the following combinations of vectors. The different types of transfected cells (and non-transfected control cells) are designated A-F as shown below, and the curves representing the LIF-binding assay data for each transfected or control cell type are also labeled A-F in Figure 1.

(A) B10G (the gp130 encoding vector described in example 3) and pHLIFR-65 (the LIF-R encoding vector described in example 3)

(B) pHLIFR-65 and control vector CAV (a control vector that does not encode LIF-R or gp130; controls for plasmid dilution so that results can be more accurately compared with those of COS-7 cells co-transfected with both a gp130 encoding vector and an LIF-R encoding vector)

(C) B10G and pHLIFR-65; transfected cells were preincubated with non-radiolabeled oncostatin M before incubation with ^{125}I-LIF

(D) pHLIFR-65 and CAV; transfected cells were preincubated with non-radiolabeled oncostatin M before incubation with ^{125}I-LIF

(E) non-transfected COS-7 cells (control)

(F) B10G and CAV

The assay was performed by a phthalate oil separation method essentially as described by Dower et al., *J. Immunol. 132*:751 (1984) and Park et al., *J. Biol. Chem. 261*:4177 (1986). Briefly, the COS-7 host cells were released from 10 cm tissue culture plates two days after transfection by incubation in non-enzymatic cell dissociation buffer (Sigma) at 37°C for 30-60 minutes. Cells were then washed with the above-described binding medium and resuspended in binding medium at 5×10^6 cells/ml. 50μl aliquots of the cells were incubated with serial dilutions of ^{125}I-LIF at room temperature for one hour with agitation (in the presence or absence of a 200-fold excess of unlabeled LIF) in a total volume of 150μl. The unlabeled LIF allowed for calculation of the non-specific background binding of LIF. Duplicate aliquots (60μl) of each incubation mixture were then transferred to a polyethylene centrifuge tube containing a phthalate oil mixture comprising 1.5 parts dibutylphthalate to 1 part bis(s-ethylhexyl)phthalate.

The cells were separated from unbound ^{125}I-LIF by centrifugation for five minutes at 15,000 x g in an Eppendorf microfuge. The centrifuge tubes were cut to separate the pellet of cells (containing bound ^{125}I-LIF) from the supernatant containing unbound ^{125}I-LIF. The radioactivity in both parts was then determined on a gamma counter. The determinations of both cell-bound and unbound radioactivity from the two 60μl aliquots were averaged for subsequent calculations.

The results are presented in Figure 1 as standard Scatchard transformations of the biological data. The data are reported as the ratio of molecules of ^{125}I-LIF bound per cell, to free ^{125}I-LIF molecules (y-axis) versus molecules of ^{125}I-LIF bound per cell (x-axis). The dissociation constants (K_D) are shown in Figure 1, along with the number of LIF-binding sites per cell. Since a saturating amount of radiolabeled LIF was offered, the number of molecules of radiolabeled LIF bound per cell is considered equivalent to the number of LIF binding sites per cell.

As shown by curve A of Figure 1, COS-7 cells co-transfected with a gp130 encoding vector (B10G) and an LIF-R encoding vector (pHLIFR-65) demonstrated high affinity LIF binding (K_D=9×10^{-10}M). When these same co-transfected COS-7 cells were preincubated with non-radiolabeled oncostatin M before incubation with ^{125}I-LIF (curve C), binding of LIF was greatly reduced (K_D=4.2×10^{-9}M). Oncostatin M thus competes with LIF for binding sites on these transfected cells.

COS-7 cells transfected with a vector encoding the single-polypeptide chain LIF-R (pHLIFR-65) and with the control vector CAV bound LIF (curve B; K_D=2.4x10^{-9}M), but with lower affinity than the cells producing both gp130 and LIF-R. The COS-7 cells display endogenous high affinity simian LIF receptors (curve E: K_D about 3x10^{-11}M). Transfection with pHLIFR-65 (encoding the single polypeptide LIF-R) results in display of additional low affinity LIF receptors (K_D=2.4x10^{-9}M; curve B, site 2) as well as the simian LIF receptors K_D=3.3x10^{-11}M; curve B, site 1).

When the COS-7 cells transfected with pHLIFR-65 and CAV were preincubated with non-radiolabeled oncostatin M before incubation with ^{125}I-LIF (CURVE D), binding of LIF to the LIF-R expressed by pHLIFR-65 was essentially unchanged compared to the same transfected cells not preincubated with oncostatin M. Oncostatin M thus does not compete with LIF for binding to the single polypeptide chain LIF-R. However, the binding of LIF to the endogenous simian high affinity LIF-R on the COS-7 cells was competed.

The COS-7 cells co-transfected with the gp130 encoding vector and the CAV control vector (curve F) did not bind LIF in any measurable amount above the amount of binding to the non-transfected COS-7 cells (curve E).

Example 2

Assay to Detect Binding of Oncostatin M

Oncostatin M may be purified from cells in which the protein is naturally found, or from cells transformed with an expression vector encoding oncostatin M. One source of oncostatin M is phorbol ester-treated U937 cells, as described by Zarling et al., *PNAS U.S.A.* 83:9739 (1986). Purification of recombinant oncostatin M is described by Linsley et al., *J. Biol. Chem.* 264:4282-4289 (1989), which is hereby incorporated by reference in its entirety.

Preferably, oncostatin M is produced in yeast cells transformed with a suitable expression vector. A DNA sequence encoding a signal sequence (e.g., a yeast alpha-factor leader sequence) may be fused to the N-terminus of the oncostatin M encoding DNA sequence to promote secretion of the protein from the host cells. The protein when initially produced may also comprise an N-terminal identification leader (e.g., a "flag" sequence such as Asp-Tyr-Lys-Asp_4-Lys) as described by Hopp et al., *Bio/Technology* 6:1204 (1988). The flag sequence is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling facile purification of the expressed recombinant protein. This sequence is also specifically cleaved by bovine mucosal enterokinase at the residue immediately following the Asp-Lys pairing.

Neither the signal sequence nor the flag sequence is found on the processed final oncostatin M product.

Oncostatin M may be radiolabeled using any suitable conventional procedure, such as the radioiodination procedure employed to radiolabel LIF in Example 1. The radio-iodination of oncostatin M has also been described by Linsley et al., *supra*.

The resulting radiolabeled oncostatin M may be substituted for radiolabeled LIF (using the same concentrations and other reaction parameters) in the assay procedures described in Example 1 in order to detect proteins and cells that bind oncostatin M. An assay for binding of ^{125}I-oncostatin M to cells is also described in Linsley et al., *supra*.

The results of one oncostatin M binding assay are shown in Figure 2. COS-7 cells transfected with vector(s) encoding gp130 or LIF-R were assayed for the ability to bind oncostatin M. In separate transfections, COS-7 cells were transfected with the following combinations of vectors. The different types of transfected cells (and non-transfected control cells) are designated A-E as shown below, and the corresponding curves representing the oncostatin M binding assay data for each cell type are also labeled A-E in Figure 2.

(A) B10G (the gp130 encoding vector described in example 3) and pHLIFR-65 (the LIF-R encoding vector described in example 3)

(B) B10G and pHLIFR-65; transfected cells were preincubated with non-radiolabeled LIF before incubation with ^{125}I-oncostatin M

(C) pHLIFR-65 and CAV (a control vector that does not encode LIF-R or gp130; controls for plasmid dilution so that results can be more accurately compared with those of COS-7 cells co-transfected with both a gp130 encoding vector and an LIF-R encoding vector)

(D) non-transfected COS-7 cells (control)

(E) B10G and CAV

The assay was performed by the phthalate oil separation method described in example 1 (but substituting oncostatin M for LIF). The results are presented in Figure 2 as standard Scatchard transformations of the biological data. The data are reported as the ratio of molecules of ^{125}I-oncostatin M bound per cell, to free ^{125}I-oncostatin M

molecules (y-axis) versus molecules of ^{125}I-oncostatin M bound per cell (x-axis). The dissociation constants (K_D) are shown in Figure 2, along with the number of oncostatin M-binding sites per cell. Since a saturating amount of radiolabeled oncostatin M was offered, the number of molecules of radiolabeled oncostatin M bound per cell is considered equivalent to the number of oncostatin M binding sites per cell.

As shown by curve A in Figure 2, COS-7 cells co-transfected with a gp130 encoding vector (B10G) and an LIF-R encoding vector (pHLIFR-65) demonstrated the ability to bind oncostatin M with high affinity (K_D-2.4x10^{-10}M).

COS-7 cells co-transfected with a vector encoding the single-polypeptide chain LIF-R (pHLIFR-65) and with the control vector CAV (curve C) did not bind oncostatin M in any significant amount above that bound by the non-transfected COS-7 cells (curve D).

COS-7 cells co-transfected with pHLIFR-65 and B10G and preincubated with non-radiolabeled LIF before incubation with ^{125}I-oncostatin M (curve B) did not bind oncostatin M in any measurable amount above that bound by the non-transfected COS-7 cells. LIF thus competes with oncostatin M for binding sites on the recombinant cells.

The experimental conditions of this assay (the results of which are shown in Figure 2) were not appropriate for accurate detection of low affinity oncostatin M receptors. Thus, a separate experiment (phthalate oil separation method) was conducted to compare oncostatin M binding by COS-7 cells transfected with B10G alone (no CAV control vector) with oncostatin M binding to non-transfected COS-7 cells. Non-transfected COS-7 cells assayed as a control demonstrated a small number of high affinity oncostatin M receptors (K_D=3.6x10^{-10}M). The cells transfected with B10G demonstrated additional low affinity binding of oncostatin M (K_D=7.7x10^{-9}M). The results of this oncostatin M binding assay are shown in Figure 3 as Scatchard transformations of the biological data. The data are reported as the ratio of molecules of ^{125}I-oncostatin M bound per cell, to free ^{125}I-oncostatin M molecules (y-axis) versus molecules of ^{125}I-oncostatin M bound per cell (x-axis). The scale in Figure 3 differs from that of Figures 1 and 2 so that the difference in oncostatin M binding by the gp130-producing cells compared to the control cells can be more readily visualized.

Disorders mediated by oncostatin M thus may be treated by administering gp130 or a fragment thereof. Receptors comprising both gp130 and LIF-R are preferred for use in treating such conditions, however, in view of the higher affinity of such receptors for oncostatin M compared to the affinity of gp130 alone for oncostatin M. gp130 also may be employed as an oncostatin M-binding reagent in diagnostic and research assays.

Example 3

Preparation of a Recombinant Fusion Protein Designated LIF-R-Linker-gp130

A recombinant receptor protein of the present invention is prepared by the following procedure. The receptor comprises an LIF-R fragment at the N-terminus attached to a gp130 fragment through a polypeptide linker. The polypeptide linker is of the formula $(Gly_4Ser)_8$. An oligonucleotide encoding a portion of the linker sequence, i.e., the sequence $Ser(Gly_4Ser)_6Gly$ is synthesized by any of the conventional known procedures for oligonucleotide synthesis. The DNA and encoded amino acids sequences of the double-stranded oligonucleotide are as follows:

SEQ ID NO: 7

```
   Bam HI

5' GATCC GGT GGA GGT GGT TCT GGT GGA GGT GGT TCA GGT GGT GGA GGA TCA
     3' G CCT CCT CCA CCA AGA CCA CCT CCA CCA AGT CCA CCA CCT CCT AGT
      Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser

                                                          BspMII  XbaI

GGA GGT GGT GGA TCA GGT GGA GGA GGT TCT GGA GGT GGA GGT TCC GGA T 3'
CCT CCA CCA CCT AGT CCA CCT CCT CCA AGA CCT CCA CCT CCA AGG CCT AGATC
5'
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
```

The remaining portion of the linker is added during vector construction as described below. This oligonucleotide as well as those discussed below may be synthesized on an automated DNA synthesis machine such as those available from Biosearch, Inc., San Rafael, California or Applied Biosystems.

The linker encoding oligonucleotide is cloned into a vector that preferably contains multiple restriction endonuclease cleavage sites that may be employed for inserting the sequences encoding LIF-R and gp130 on either side of, and in the same reading frame as, the sequence encoding the linker. One such vector is designated pBLUESCRIPT SK® which is available from Stratagene Cloning Systems, La Jolla, California. This plasmid vector is replicable in *E. coli* and contains a polylinker segment that includes 21 unique restriction sites. The plasmid is digested with the restriction enzymes BamHI and XbaI and the linker-encoding oligonucleotide is ligated into the vector using conventional techniques. A recombinant vector containing the inserted oligonucleotide sequence is identified by restriction endonuclease analysis and

sizing by gel electrophoresis. A DNA sequence encoding LIF-R is inserted into the pBLUESCRIPT SK® vector upstream of the linker- encoding oligonucleotide and a DNA sequence encoding gp130 is inserted downstream of the linker sequence. cDNA molecules encoding soluble fragments of LIF-R and gp130 were isolated and amplified using the well known polymerase chain reaction (PCR) procedure. The following oligonucleotides were synthesized for use in the PCR procedures:

SEQ ID NO: 8 (Oligonucleotide No. 1)

SalI

5' GATATGTCGACGATGATGGATATTTACGTATGTTTG 3'

SEQ ID NO: 9 (Oligonucleotide No. 2)

3' CATACATACACCACTGTTTCCTTTTAAGACCTCCTCCACCTAGGTACG 5'

BamHI

SEQ ID NO: 10 (Oligonucleotide No. 3)

BspMII

5' CGCGTCCGGAGGAGGTGGATCTGAACTTCTAGATCCATGTGGTTATATC 3'

SEQ ID No. 11 (Oligonucleotide No. 4)

3' CAAACGAGTTCCTCTTTAACTTATCCGCCGGCGTACG 5'

Oligonucleotides 1 and 2 are used in a PCR reaction to isolate a soluble fragment of LIF-R. The template employed in the reaction is the human LIF-R cDNA cloned as described by Gearing et al. *supra*. The DNA and encoded amino acid sequences of the cDNA clone are represented in SEQ ID NO: 5. The cloning vector which contains this human LIF-R cDNA clone was deposited in *E. coli* host cells with the American Type Culture Collection, Rockville, Maryland, U.S.A. on December 11, 1990, under the name pHLIFR-65 (ATCC Accession Number 68491). The deposit was made under the conditions of the Budapest Treaty. The 5' primer is oligonucleotide No. 1, which includes a DNA sequence encoding the first 8 amino acids of the signal sequence of LIF-R and also comprises upstream sequences that introduce a Sal 1 restriction endonuclease cleavage site. Oligonucleotide No. 1 is capable of annealing to the (-) strand that is complementary to nucleotides 179-202 of SEQ ID NO: 5. The 3' primer is oligonucleotide No. 2, which contains a sequence

complementary to nucleotides 2651-2677 of SEQ ID NO: 5 (i.e., includes anti-sense nucleotides encoding the last nine amino acids of the extracellular domain of LIF-R). Immediately downstream of the LIF-R encoding sequence, oligonucleotide No. 2 contains a sequence encoding $(Gly)_4$ Ser, and also introduces a BamHI restriction endonuclease cleavage site.

A PCR reaction employing oligonucleotides Nos. 1 and 2 thus isolates and amplifies a DNA sequence encoding an LIF-R fragment containing the entire signal sequence and the entire extracellular domain but lacking the transmembrane region and the extracellular domain. The $(Gly)_4$ Ser sequence attached to the 3' terminus of the LIF-R fragment is part of the polypeptide linker in the final construct.

Any suitable PCR procedure may be employed. One such procedure is described in Sarki et al., *Science 239*:487 (1988). Another is described in *Recombinant DNA Methodology*, Wu et al., eds., Academic Press Inc., San Diego (1989), pp. 189-196. In general, PCR reactions involve combining the 5' and 3' nucleotides with the template DNA and each of the four deoxynucleoside triphosphates in a suitable buffered solution. The solution is heated, (e.g. from 95° to 100°C) to denature the double-stranded DNA template and is then cooled before addition of a DNA polymerase enzyme. Multiple cycles of the reactions are carried out in order to amplify the desired DNA fragment.

An example of a suitable PCR procedure is as follows. All temperatures are in degrees centigrade. The following PCR reagents are added to a 1.5 ml Eppendorf microfuge tube: 10 µl of 10X PCR buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.3 at 25°C, 25 mM $MgCl_2$, and 1 mg/ml gelatin) (Perkin-Elmer Cetus, Norwalk, CN), 10 µl of a 2 mM solution containing each dNTP (2 mM dATP, 2 mM dCTP, 2 mM dGTP and 2 mM dTTP), 2.5 units (0.5 µl of standard 5000 units/ml solution) of *Taq* DNA polymerase (Perkin-Elmer Cetus), 50 ng of template DNA, 5 µl of a 20 µM solution of each of oligonucleotide primers 1 and 2, and 74.5 µl water to a final volume of 100 µl. The final mixture is then overlaid with 100 µl parafin oil. PCR is carried out using a DNA thermal cycler (Ericomp, San Diego, CA) by initially denaturing the template at 94° for 90 seconds, reannealing at 55° for 75 seconds and extending the cDNA at 72° for 150 seconds. PCR is carried out for an additional 20 cycles of amplification using a step program (denaturation at 94°, 25 sec; annealing at 55°, 45 sec: extension at 72°, 150 sec.), followed by a 5 minute extension at 72°.

The sample is removed from the parafin oil and DNA extracted by phenolchloroform extraction and spun column chromatography over G-50 (Boehringer Mannheim). A 10 µl aliquot of the extracted DNA is separated by electrophoresis on

1% SeaKem™ agarose (FMC BioProducts, Rockland, ME) and stained with ethidium bromide to confirm that the DNA fragment size is consistent with the predicted product.

The PCR-amplified cDNA products are then digested with SalI and BamHI restriction enzymes using standard procedures. The SalI/BamHI restriction fragment is then separated by gel electrophoresis, e.g., on a 1.2% Seaplaque™ low gelling temperature (LGT) agarose, and the band representing the desired fragment is isolated. The fragment is inserted into a vector encoding the desired fusion protein as described below.

A plasmid vector containing human gp130 cDNA was deposited in *E. coli* strain DH5α host cells with the American Type Culture Collection, Rockville, Maryland under the name B10G/pDC303 (DH5α) on November 14, 1991 and assigned ATCC Accession No. 68827. The deposit was made under the conditions of the Budapest Treaty. The DNA and encoded amino acid sequences of this cloned cDNA are shown in SEQ ID NO: 1.

Oligonucleotides 3 and 4 are employed in the polymerase chain reaction procedure to amplify and isolate a DNA fragment encoding Ser(Gly)$_4$ Ser followed by amino acids 1 to 597 of SEQ ID NO: 1 (the entire extracellular domain of the mature gp130 protein). The 5' primer, oligonucleotide No. 3, includes nucleotides 310 to 336 of SEQ ID NO: 1, which encode the first nine amino acids of the mature gp130 protein. This nucleotide sequence is capable of annealing to the (-) strand that is complementary to nucleotides 310 to 336 of SEQ ID NO: 1. Oligonucleotide No. 3 also encodes a Ser(Gly)$_4$ Ser sequence directly upstream of (and in the same reading frame as) the gp130 sequence, and further positions a BspMII restriction endonuclease cleavage site near the 5' terminus of the Ser(Gly)$_4$ Ser -encoding sequence.

The 3' primer, oligonucleotide No. 4, includes a sequence complimentary to nucleotides 2080 to 2100 of SEQ ID NO: 1, i.e., includes anti-sense nucleotides encoding the last seven amino acids of the gp130 extracellular domain. Oligonucleotide No. 4 positions a stop codon immediately after the gp130 sequence and also inserts a NotI restriction site downstream. Following amplification of the gp130 fragment by PCR, the PCR reaction products are digested with BspMII and NotI and the desired fragment is isolated.

The above-described LIF-R, Ser(Gly$_4$Ser)$_6$Gly linker, and gp130 encoding fragments are assembled into a single DNA sequence as follows. The Ser(Gly$_4$Ser)$_6$Gly linker fragment is excised from the pBLUESCRIPT SK® vector by digestion with BamHI and BspMII. The linker fragment is then ligated to the 3' end of the LIF-R fragment (cleaved at its 3' terminus after the Gly$_4$Ser sequence with BamHI). The ligation is conducted under conventional conditions. The 3' end of the

linker fragment is ligated to the BspMII-cleaved 5' end of the gp130 fragment. The resulting DNA fragment encodes a receptor of the present invention comprising (from 5' to 3') the signal sequence and extracellular domain of LIF-R attached to a $(Gly_4Ser)_8$ polypeptide linker which is attached to the mature coding sequence of the gp130 extracellular domain.

This DNA fragment may be inserted into any suitable cloning and/or expression vector. For example, the pBLUESCRIPT SK® vector may be digested with SalI and NotI and the ligated DNA fragment inserted therein. *E. coli* cells are then transformed with the recombinant vector by conventional procedures.

In an alternative procedure, the pBLUESCRIPT SK® vector containing the $Ser(Gly_4Ser)_6Gly$ linker sequence is digested with SalI and BamHI and the above described LIF-R-encoding fragment is inserted therein. The resulting vector is then digested with BspMII and NotI and the gp130-encoding fragment is then inserted to form the DNA sequence encoding the receptor of the present invention. The cloned receptor-encoding DNA fragment may be excised and inserted into any suitable expression vector (chosen according to the type of host cell that is desired) using conventional procedures. Host cells transformed with the recombinant expression vector are cultivated to produce the receptor protein. Mammalian host cells are generally preferred for producing the recombinant receptor fusion proteins of the present invention.

The receptor-encoding construct may be excised by SalI and NotI digestion and inserted into a vector suitable for use in mammalian host cells. One suitable vector is designated pDC406. cDNA molecules inserted at the SalI site of this vector are transcribed and translated using regulatory elements derived from HIV and adenovirus. pDC406 contains origins of replication derived from SV40, Epstein-Barr virus and pBR322. The pDC406 vector into which interleukin-1 receptor cDNA has been cloned has been deposited with the American Type Culture Collection, Rockville, Maryland USA under accession number CRL10478. The interleukin-1 receptor cDNA may be excised from the vector using conventional techniques and replaced with the receptor encoding DNA of the present invention prepared above. pDC406 is a derivative of HAV-EO described by Dower et al., *J. Immunol.* *142*:4314 (1989). pDC406 differs from HAV-EO by the deletion of the intron present in the adenovirus 2 tripartite leader sequence in HAV-EO.

Examples of suitable mammalian cells for expressing a receptor fusion protein include CV-1 cells (ATCC CCL70) and COS-7 cells, (ATCC CRL 1651) both derived from monkey kidney. Another monkey kidney cell line CV-1/EBNA (ATCC CRL 10478) was derived by transfection of the CV-1 cell line with a gene encoding Epstein-

Barr virus nuclear antigen-1 (EBNA-1) and with a vector containing CMV regulatory sequences. See McMahan et al., *EMBO J. 10*:2821 (1991). The EBNA-1 gene allows for episomal replication of expression vectors, such as HAV-EO or pDC406, that contain the EBV origin of replication.

Example 4

Preparation of a Recombinant Receptor Fusion Protein Designating gp130-Linker-LIF-R

This receptor of the present invention differs from that of Example 3 in that the LIF-R polypeptide (which was the 5' polypeptide in the receptor of Example 3) is now the 3' polypeptide. The following oligonucleotides were synthesized for use in preparing the fusion protein:

SEQ ID NO: 12
5' GATATGTCGACAAGATGTTGACGTTGCAGACTTGG 3' (oligonucleotide no. 5)

SEQ ID NO: 13
3' CAAACGAGTTCCTCTTTAACTTCCTCCTCCACCTAGGTACG 5' (oligonucleotide no. 6)

SEQ ID NO: 14
5' CGCGTCCGGAGGAGGTGGTAGCCAGAAAAAGGGGCTCCTCATG 3' (oligonucleotide no. 7)

SEQ ID NO: 15
3' CATACATACACCACTGTTTCCTTTTAAGAATCGCCGGCGTACG 5' (oligonucleotide no. 8)

Oligonucleotides 5 and 6 are employed in a polymerase chain reaction procedure to isolate a fragment of gp130. The 5' primer (oligonucleotide number 5) includes nucleotides 244 to 264 of SEQ ID NO: 1, (the sequence encoding the first seven amino acids of the gp130 signal sequence). Oligonucleotide number 5 also includes a sequence that introduces an upstream SalI site. This nucleotide sequence is capable of annealing to the (-) strand that is complementary to nucleotides 244 to 264 of SEQ ID NO: 1. The 3' primer (oligonucleotide number 6) includes a sequence complementary to nucleotides 2080 to 2100 of SEQ ID NO: 1, i.e. includes antisense nucleotides encoding the last seven amino acids of the gp-130 extracellular domain.

Oligonucleotide number 6 also encodes a Gly4Ser sequence immediately 3' to (and in phase with) the gp130 sequence, and also inserts a downstream BamHI site.

A PCR reaction is conducted as described in Example 3 but employing oligonucleotides 5 and 6 on the gp130 cDNA template. A DNA sequence encoding a gp130 fragment that includes the 5' signal sequence and the entire extracellular domain, but none of the transmembrane region or the cytoplasmic domain, is isolated by the PCR reaction. A Gly4Ser sequence is fused to the 3' terminus of the gp130 fragment. The PCR reaction products are digested with SalI and BamHI and the desired fragment is isolated.

An LIF-R fragment is isolated and amplified by a PCR reaction employing oligonucleotides 7 and 8. The 5' primer (oligonucleotide number 7) includes nucleotides 311 to 331 of SEQ ID NO: 5, which encode the first seven amino acids of the mature LIF-R protein. This nucleotide sequence is capable of annealing to the (-) strand complementary to nucleotides 311 to 331 of SEQ ID NO: 5. Oligonucleotide number 7 also encodes a Gly4Ser sequence fused to the 5' end of the LIF-R sequence, and inserts an upstream BspMII site. The 3' primer (oligonucleotide number 8) is complementary to nucleotides 2651 to 2677 of SEQ ID NO: 5 (which encode the last nine amino acids of the LIF-R extracellular domain.) Oligonucleotide number 8 also adds a stop codon at the 3' end of the LIF-R sequence, and inserts a Not I site downstream. The PCR reaction products are digested with BspMII and NotI and the desired fragment is isolated.

A DNA sequence encoding the desired receptor protein is prepared by ligating the BamHI site of the gp130 fragment prepared above to the BamHI site at the 5' terminus of the linker fragment described in Example 3. Likewise the C-terminus of the linker encoding fragment is ligated at the BspMII site to the complementary site of the LIF-R encoding fragment prepared above. The resulting DNA fragment may be cloned into an expression vector using procedures described in Example 3. The receptor encoded by the isolated DNA fragment comprises (from the N-terminus to the C-terminus) the signal sequence and extracellular domain of gp130 attached to a $(Gly_4Ser)_8$ polypeptide linker which is attached to the mature coding sequence of the LIF-R extracellular domain.

Example 5

Receptor Fusion Protein Comprising LIF-R Attached to gp-130 Through An Fc Polypeptide Linker

A receptor prepared in accordance with the following procedures is depicted in Figure 4. The following oligonucleotides were synthesized for use in preparing the receptor fusion protein:

SEQ ID NO: 16

3' CATACATACACCACTGTTTCCTTTTAAGACTCGGGTCTAGATACG 5' (oligonucleotide no. 9)

SEQ ID NO: 17

3' CAAACGAGTTCCTCTTTAACTTCTCGGGTCTAGATACG 5' (oligonucleotide no. 10)

An LIF-R encoding DNA sequence is isolated and amplified in a PCR reaction using oligonucleotides 1 and 9. Oligonucleotide number 1 (the 5' primer) inserts an upstream SalI site and has been described in Example 3. The 3' primer is oligonucleotide number 9 which includes a sequence complementary to nucleotides 2651 to 2677 of SEQ ID NO: 5, i.e., includes antisense nucleotides encoding the last nine amino acids of the extracellular domain of LIF-R. Oligonucleotide number 9 also inserts a downstream BglII site. The PCR reaction products are digested with SalI and BglII, and the desired LIF-R encoding DNA fragment is isolated by gel electrophoresis using conventional procedures. Due to the presence of an internal BglII site in the LIF-R sequence, the BglII digestion should be carried out under conditions that effect partial digestion.

A gp130 encoding DNA fragment is isolated and amplified by PCR reaction using oligonucleotides 5 and 10. The 5' primer (oligonucleotide number 5) inserts an upstream SalI site and has been described above in Example 4. The 3' primer is oligonucleotide number 10, which includes a sequence complementary to nucleotides 2080 to 2100 of SEQ ID NO: 1, i.e., includes antisense nucleotides encoding the last seven amino acids of the gp130 extracellular domain. Oligonucleotide number 10 also inserts a downstream BglII site. The PCR reaction products are digested with SalI and BglII, and the desired gp130 encoding DNA fragment is isolated by gel electrophoresis using conventional techniques.

cDNA encoding a single chain polypeptide derived from the Fc region of a human IgG1 antibody has been cloned into the above-described pBLUESCRIPT SK®

vector to produce a recombinant vector designated hIgG1Fc. A unique BglII site is positioned near the 5' end of the inserted Fc encoding sequence. An SpeI site is immediately downstream of the stop codon. The DNA and encoded amino acid sequences of the cloned Fc cDNA are presented in SEQ ID NO: 3 and SEQ ID NO: 4.

The Fc polypeptide encoded by the cDNA extends from the N-terminal hinge region to the native C-terminus, i.e., is an essentially full-length antibody Fc region. Fc fragments, e.g., those that are truncated at the C-terminal end, also may be employed. The fragments should contain multiple cysteine residues (at least the cysteine residues in the hinge reaction). The antibody from which the Fc polypeptide is derived is preferably of the same species as the patient to be treated with the fusion protein prepared therefrom..

Plasmid hIgG1Fc is digested with BglII and SalI and the BglII/SalI LIF-R fragment prepared above is ligated into the vector by conventional techniques. The Fc encoding sequence is positioned downstream of, and in the same reading frame as, the LIF-R sequence. In a separate reaction, the above-described SalI/BglII fragment of gp130 is also inserted into the same vector. Plasmid vectors containing the desired DNA insert are identified by restriction endonuclease digestion analysis, using convention techniques.

The cloned DNA segment encoding the LIF-R-Fc fusion polypeptide may be excised from the pBLUESCRIPT SK® vector by digestion with SalI and NotI. Likewise, the DNA segment encoding the gp130-Fc fusion polypeptide may be excised by SalI/NotI digestion. Each excised DNA segment is inserted into an appropriate expression vector, depending on the type of host cell that is desired. One suitable expression vector is the plasmid pDC406, which may be transformed into mammalian host cells as described in Example 3.

In one embodiment of the invention, an expression vector encoding the LIF-R-Fc fusion and a second expression vector encoding the gp130-Fc fusion are co-transfected into the desired host cells. Two separate recombinant polypeptides are thus produced in the host cells. The first polypeptide comprises the Fc polypeptide fused in frame to the C-terminus of the gp130 fragment. The second polypeptide comprises the Fc polypeptide fused in frame to the C-terminus of the LIF-R fragment. Disulfide bonds that form between the two Fc regions covalently link the two separate fusion polypeptides into a receptor protein of the present invention.

Alternatively, the LIF-R-Fc and gp130-Fc polypeptides may be separately transformed into host cells (as opposed to co-transfection into the same host cell.) The two polypeptides are purified from the host cells and then combined in a suitable

buffered solution, whereupon interchain disulfide bonds form between the two Fc regions.

The receptor protein may be purified using any of a number of conventional protein purification techniques. Since antibody Fc regions bind to protein A and protein G, affinity chromatography employing protein A or protein G attached to an insoluble support material may be employed in the purification process. In one procedure, one liter of culture supernatant containing the receptor is passed over a solid phase protein G column, and the column is then washed thoroughly with phosphate-buffered saline (PBS). The adsorbed Fc-containing fusion protein is eluted with 50 mM glycine buffer, pH 3 and brought to pH 7 with 2 M Tris buffer, pH 9. Further purification may involve immunoaffinity column(s), e.g., affinity columns having LIF or OSM bound thereto.

Example 6

Preparation of Monoclonal Antibodies Directed against a Receptor

Preparations of a purified receptor protein of the present invention, or transfected COS cells expressing high levels of the receptor, are employed to generate monoclonal antibodies against the receptor using conventional techniques, for example, those disclosed in U.S. Patent 4,411,993. To immunize mice, a receptor immunogen is emulsified in complete Freund's adjuvant and injected subcutaneously in amounts ranging from 10-100μg into Balb/c mice. Ten to twelve days later, the immunized animals are boosted with additional immunogen emulsified in incomplete Freund's adjuvant and periodically boosted thereafter on a weekly to biweekly immunization schedule. Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision for testing by dot-blot assay (antibody sandwich) or ELISA (enzyme-linked immunosorbent assay). Other assay procedures are also suitable. Following detection of an appropriate antibody titer, positive animals are given an intravenous injection of antigen in saline. Three to four days later, the animals are sacrificed, splenocytes harvested, and fused to the murine myeloma cell line NS1. Hybridoma cell lines generated by this procedure are plated in multiple microtiter plates in a HAT selective medium (hypoxantine, aminopterin, and thymidine) to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

Hybridoma clones thus generated can be screened by ELISA for reactivity with the receptor protein, for example, by adaptations of the techniques disclosed by Engvall et al., *Immunochem* 8.871 (1971) and in U.S. Patent 4,704,004. Positive clones are then injected into the peritoneal cavities of syngeneic Balb/c mice to produce ascites

containing high concentrations (greater than 1 mg/ml) of anti-receptor monoclonal antibody. The resulting monoclonal antibody can be purified by ammonium sulfate precipitation followed by gel exclusion chromatography, and/or affinity chromatography based on binding of antibody to Protein A of *Staphylococcus aureus*.

Example 7

Heterodimeric Receptor

An expression vector encoding a fragment of the human LIF-R extracellular domain fused to a polypeptide derived from the Fc region of an antibody was constructed as follows. A second expression vector encoding a fragment of the human gp130 extracellular domain fused to an Fc polypeptide also was constructed.

Plasmid pHLIF-R-65 (ATCC 68491), which contains human LIF-R cDNA in expression vector pDC303 as described in example 3, was digested with the restriction enzymes *Asp718* and *XmnI*. *Asp718* cleaves the vector upstream of the LIF-R cDNA insert. *XmnI* is a blunt cutter that cleaves within the codon for amino acid number 702 (Asp) of SEQ ID NO:5, upstream of the transmembrane region. The desired *Asp718/XmnI* fragment (about 2,444 bp in length) was separated by electrophoresis on an agarose gel and purified by conventional procedures using an Elutip column.

A recombinant vector designated hIgG1Fc, comprising cDNA encoding a single chain polypeptide derived from the Fc region of a human IgG1 antibody in a pBLUESCRIPT SK® vector was described in example 5. The DNA and encoded amino acid sequences of the cloned Fc cDNA are presented in SEQ ID NO:3 and SEQ ID NO:4. A polylinker region comprising a number of restriction sites is positioned immediately upstream of the Fc cDNA.

Plasmid hIgG1Fc was digested with *Asp718* and *SnaI*, which cleave within the pollylinker upstream of the Fc sequence. The *Asp718/XmnI* LIF-R fragment prepared above was ligated into the cleaved hIgG1Fc vector by conventional techniques. *SnaI* and *XmnI* both produce blunt ends, which will ligate together. In the resulting recombinant vector, the Fc encoding sequence is positioned downstream of, and in the same reading frame as, the LIF-R sequence. The encoded LIF-R/Fc fusion protein comprises amino acids -44 to 702 of SEQ ID NO:5 (LIF-R), followed by six amino acids constituting a peptide linker encoded by the polylinker segment of plasmid hIgG1Fc, followed by amino acids 1-232 of SEQ ID NO:3 (Fc). *E. coli* cells were transformed with the ligation mixture and plasmids were isolated therefrom by standard procedures. Plasmid vectors containing the desired DNA insert were identified by restriction endonuclease digestion analysis.

The cloned DNA segment encoding the LIF-R/Fc fusion polypeptide was excised from the recombinant vector by digestion with *Asp718* and *NotI*. The *NotI* enzyme cleaves the vector in a polylinker region just downstream of the Fc cDNA insert. The excised DNA segment (3.2 kb) is inserted into an appropriate expression vector, depending on the type of host cell that is desired. One suitable expression vector is pCAV/NOT, a mammalian expression vector described in PCT application WO 90/05183.

pCAV/NOT was cleaved with *Asp718* and *NotI*, both of which cleave in the multiple cloning site. The LIF-R/Fc-encoding *Asp718/NotI* DNA fragment prepared above was ligated into the vector.

An expression vector encoding a soluble gp130/Fc fusion protein was constructed as follows. Recombinant vector B10G/pDC303 (ATCC 68827) comprising human gp130 cDNA (described in example 3) was digested with EcoR1, and the resulting 5' overhang was rendered blunt using T4 DNA polymerase. The recognition site for EcoR1 comprises nucleotides 2056-2061 of SEQ ID NO:1. The EcoR1-digested vector was then cleaved with XhoI, which cleaves in the vector upstream of the gp130 cDNA insert.

Plasmid hIgG1Fc, comprising Fc polypeptide-encoding cDNA as described above, was digested with StuI (a blunt cutter) and NotI, which cleave upstream and downstream, respectively, of the inserted Fc cDNA. The (EcoR1)/XhoI gp130 fragment isolated above was ligated to the Fc-containing fragment and to XhoI/NotI-digested vector SF CAV/NOT. The mammalian expression vector SF CAV/NOT is essentially identical to SF CAV (ATCC 68922) but contains a Not1 site. SF CAV/NOT also is essentially identical to pCAV/NOT, described in PCT application WO 90/05183, except that a segment of the adenovirus-2 tripartite leader (TPL) containing a cryptic promoter functional in bacteria has been deleted. Protein expression from the cryptic promoter is potentially disadvantageous for preparing and isolating a desired recombinant plasmid in bacterial cells.

E. coli cells were transformed with the ligation mixture, plasmids were isolated therefrom by conventional procedures, and the desired recombinant plasmids were identified by restriction analysis. The gp130/Fc fusion protein encoded by the desired recombinant vector comprises (from N- to C-terminus) amino acids -22 to 582 of SEQ ID NO:2 (gp130), followed by 7 amino acids constituting a peptide linker encoded by the polylinker segment of plasmid hIgG1Fc, followed by amino acids 1-232 of SEQ ID NO:4 (Fc).

COS-7 cells (ATCC CRL 1651) were transfected with either the LIF-R/Fc-encoding recombinant expression vector or the gp130/Fc-encoding expression vector

prepared above, or with both expression vectors. The cells were cultivated to allow expression of the soluble fusion proteins. The expressed proteins were recovered by incubating culture supernatant with Protein G Sepharose beads (available from Pharmacia) overnight at 4°C, then pelleting the beads by centrifugation. The binding of ^{125}I-labeled human oncostatin M and ^{125}I-labeled human LIF by the proteins bound to the beads was analyzed.

Binding affinity was determined by performing a variation of a standard Scatchard analysis. The binding assay procedure was similar to that described by Mosley et al. (*Cell* 59:335, 1989) except that the proteins, being soluble, are attached to Protein G Sepharose beads rather than being on the surface of the transfected cells. Briefly, in a 96-well microtiter plate, each of ten 1:2 serial dilutions of ^{125}I-LIF or ^{125}I-oncostatin M was incubated with a sample comprising the expressed proteins (bound to the beads) resuspended in RPMI 1640 containing 2.5% bovine serum albumin, 0.2% (v/v) sodium azide and 20mM Hepes, pH 7.4, for 2 hours at 4°C with agitation. Duplicate standard cold competition wells also were incubated. Centrifuge tubes containing bovine calf serum were used in place of the phthalate oil mixture-containing tubes in the separation method described by Dower et al., *J. Immunol.* 132:751 (1984) and Park et al., *J. Biol. Chem.* 261:4177 (1986) and in example 1 above. Aliquots of each incubation mixture were transferred to the tubes. After centrifugation, tubes were cut, the radioactivity counted, and processed as for standard Scatchard analysis.

Figure 7 presents Scatchard analyses of the binding of ^{125}I oncostatin M by gp130/Fc homodimers produced by the cells transfected with the gp130/Fc vector alone (upper left) and by the proteins expressed by the co-transfected cells (lower left). Scatchard analyses of the binding of ^{125}I LIF by LIF-R/Fc homodimers produced by the cells transfected with the LIF-R/Fc vector alone (upper right) and by the proteins expressed by the co-transfected cells (lower right) are also presented in figure 7. A shift toward higher affinity binding of oncostatin M by the proteins recovered from the co-transfected cells, compared to the gp130/Fc homodimer, is evident from figure 7. Likewise, the data in figure 7 indicate a shift toward higher affinity binding of LIF by the proteins recovered from the co-transfected cells, compared to the LIF-R/Fc homodimer. The shift toward higher affinity binding indicates the presence of heterodimers comprising LIF-R/Fc and gp130/Fc, and further indicates that the LIF-R and gp130 moieties are cooperating, i.e., interacting, in the binding of oncostatin M and LIF. Controls demonstrated no oncostatin M binding by LIF-R homodimers, and no LIF binding by gp130 homodimers.

Example 8

Receptors Comprising LIF-R and gp130 Polypeptides Lacking FNIII Domains

DNA sequences encoding soluble LIF-R and gp130 proteins lacking fibronectin type III (FNIII) domains were isolated and fused to an Fc-encoding sequence. Deleting the FNIII domains affords the advantage of reducing the size of the LIF-R/Fc and gp130/Fc fusion proteins. The LIF-R protein of SEQ ID NO:6 comprises three repeats of a fibronectin type III-like module in the extracellular domain. The three domains containing FNIII modules comprise amino acids 487 (Thr) to 584 (Asn), 585 (Asp) to 679 (Ala), and 680 (Pro) to 789 (Ser), respectively, of SEQ ID NO:6. gp130 also contains three FNIII domains, comprising amino acids 300 (Tyr) to 399 (Phe), 400 (Gln) to 496 (Pro), and 497 (Pro) to 597 (Glu), respectively, of SEQ ID NO:2. From one to all three of the FNIII domains may be removed from gp130 or LIF-R to reduce the size of the protein.

The FNIII domains of human LIF-R were removed by digesting the LIF-R/Fc-encoding expression vector prepared in example 7 with the restriction endonuclease Eco O 109I (isoschizomer of Dra II) and filling in the resulting overhangs using T4 DNA polymerase according to conventional procedures. The recognition site for Eco O 109I spans nucleotides 1789-1795 of SEQ ID NO:5 (LIF-R), cleaving within the codons for amino acids 8-9 of the first FNIII domain of LIF-R. The cleaved vector was then digested with BstX1 and EcoR5. The recognition site for BstX1 spans nucleotides 1048-1059 of SEQ ID NO:5 and EcoR5 (which generates blunt ends) cleaves within the polylinker upstream of the Fc sequence. The BstX1/EcoR5 fragment (comprising the 5' end of LIF-R, the vector, the entire Fc sequence, and a portion of the polylinker) and the BstX1/(Eco O 109I) LIF-R fragment were isolated and ligated together. *E. coli* cells were transformed with the ligation mixture, plasmids were isolated therefrom, and the desired recombinant plasmid was identified by restriction analysis. The resulting construct encodes a fusion protein comprising (from N- to C-terminus) amino acids -44 to 494 of SEQ ID NO:5 (LIF-R), a four amino acid spacer peptide -His-Arg-Tyr-Val- encoded by the polylinker segment, and amino acids 1-232 of SEQ ID NO:3 (Fc). The LIF-R polypeptide moiety contains the first 8 amino acids of the first FNIII domain, but lacks the remainder of the first FNIII domain and all of the second and third FNIII domains.

The FNIII domains of gp130 were removed by digesting the recombinant gp130/Fc-encoding expression vector prepared in example 7 with BstX1, then blunting the overhang using T4 DNA polymerase according to conventional procedures. The recognition site for BstX1 spans nucleotides 1231-1242 of SEQ ID NO:1 (gp130).

cleaving within the codons for amino acids 10-11 of the first FNIII domain of gp130. The cleaved vector was then digested with EcoR5, which cleaves within the polylinker upstream of the Fc sequence and generates blunt ends. The (BstX1)/EcoR5 fragment comprising the 5' end of gp130 (lacking the FNIII domains), the vector sequences, the Fc sequence, and a portion of the polylinker, was ligated. *E. coli* cells were transformed with the ligation mixture, plasmids were isolated therefrom, and the desired recombinant plasmid was identified by restriction analysis. The fusion protein encoded by the construct comprises (from N- to C-terminus) amino acids -22 to 308 of SEQ ID NO:2 (gp130), a four amino acid spacer peptide -Asn-Arg-Tyr-Val- encoded by the polylinker segment, and amino acids 1-232 of SEQ ID NO:3 (Fc). The gp130 polypeptide moiety contains the first 9 amino acids of the first FNIII domain, but lacks the remainder of the first FNIII domain and all of the second and third FNIII domains.

FNIII domains may be deleted from the gp130 component of a receptor of the present invention, from the LIF-R component, or from both. In one embodiment of the invention, COS-7 cells were co-transfected with the soluble LIF-R/Fc-encoding mammalian expression vector prepared in example 7 and the mammalian expression vector encoding a soluble gp130/Fc protein lacking the FNIII domains prepared above. Analysis of the expressed proteins by SDS-PAGE revealed a band of the molecular weight expected for the heterodimer, along with bands that include those of the molecular weight expected for the two homodimers. Scatchard analyses conducted according to the procedures described in example 7 demonstrated a shift toward higher affinity binding of LIF and oncostatin M for proteins expressed by the co-transfected cells compared to the corresponding homodimers. This result indicates the presence of heterodimers comprising LIF-R/Fc and gp130/Fc, and further indicates that the LIF-R and gp130 moieties are cooperating, i.e., interacting, in binding oncostatin M and LIF.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO:1 and SEQ ID NO:2 present the DNA sequence and encoded amino acid sequence for cloned cDNA encoding an N-terminal fragment of gp130.

SEQ ID NO:3 and SEQ ID NO:4 present the DNA sequence and encoded amino acid sequence for cloned cDNA encoding a polypeptide that corresponds to the Fc region of an IgG1 antibody.

SEQ ID NO:5 and SEQ ID NO:6 present the DNA sequence and encoded amino acid sequence for cloned cDNA encoding an N-terminal fragment of LIF-R.

SEQ ID NO:7 presents the DNA sequence of the coding strand of a chemically synthesized DNA molecule encoding a polypeptide linker used in constructing certain receptors of the present invention.

SEQ ID NO:8 - SEQ ID NO:17 present the DNA sequence of various single-stranded oligonucleotide primers employed in polymerase chain reactions to construct certain receptors of the present invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Gearing, David P.

(ii) TITLE OF INVENTION: Receptor for Oncostatin M and Leukemia Inhibitory Factor

(iii) NUMBER OF SEQUENCES: 17

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Immunex Corporation
(B) STREET: 51 University Street
(C) CITY: Seattle
(D) STATE: WA
(E) COUNTRY: USA
(F) ZIP: 98101

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Seese, Kathryn A.
(B) REGISTRATION NUMBER: 32,172
(C) REFERENCE/DOCKET NUMBER: 2607

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 206-587-0430
(B) TELEFAX: 206-587-0606

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2369 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: human placenta

(vii) IMMEDIATE SOURCE:

(B) CLONE: B10G/pDC303

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 244..2369

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 310..2369

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 244..309

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TCGAGGCGGC GCGGCCTGAG TGAAACCCAA TGGAAAAAGC ATGACATTTA GAAGTAGAAG   180

ACTTAGCTTC AAATCCCTAC TCCTTCACTT ACTAATTTTG TGATTTGGAA ATATCCGCGC   240

AAG ATG TTG ACG TTG CAG ACT TGG CTA GTG CAA GCC TTG TTT ATT TTC     288
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    -22     -20             -15                 -10

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         -5                  1               5

AGT CCT GAA TCT CCA GTT GTA CAA CTT CAT TCT AAT TTC ACT GCA GTT     384
Ser Pro Glu Ser Pro Val Val Gln Leu His Ser Asn Phe Thr Ala Val
 10                  15                  20                  25

TGT GTG CTA AAG GAA AAA TGT ATG GAT TAT TTT CAT GTA AAT GCT AAT     432
Cys Val Leu Lys Glu Lys Cys Met Asp Tyr Phe His Val Asn Ala Asn
                 30                  35                  40

TAC ATT GTC TGG AAA ACA AAC CAT TTT ACT ATT CCT AAG GAG CAA TAT     480
Tyr Ile Val Trp Lys Thr Asn His Phe Thr Ile Pro Lys Glu Gln Tyr
             45                  50                  55

ACT ATC ATA AAC AGA ACA GCA TCC AGT GTC ACC TTT ACA GAT ATA GCT     528
Thr Ile Ile Asn Arg Thr Ala Ser Ser Val Thr Phe Thr Asp Ile Ala
         60                  65                  70

TCA TTA AAT ATT CAG CTC ACT TGC AAC ATT CTT ACA TTC GGA CAG CTT     576
Ser Leu Asn Ile Gln Leu Thr Cys Asn Ile Leu Thr Phe Gly Gln Leu
     75                  80                  85

GAA CAG AAT GTT TAT GGA ATC ACA ATA ATT TCA GGC TTG CCT CCA GAA     624
Glu Gln Asn Val Tyr Gly Ile Thr Ile Ile Ser Gly Leu Pro Pro Glu
 90                  95                 100                 105

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Lys Pro Lys Asn Leu Ser Cys Ile Val Asn Glu Gly Lys Lys Met Arg
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140 145 150

CGT GAC ACC CCC ACC TCA TGC ACT GTT GAT TAT TCT ACT GTG TAT TTT 816
Arg Asp Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser Thr Val Tyr Phe
155 160 165

GTC AAC ATT GAA GTC TGG GTA GAA GCA GAG AAT GCC CTT GGG AAG GTT 864
Val Asn Ile Glu Val Trp Val Glu Ala Glu Asn Ala Leu Gly Lys Val
170 175 180 185

ACA TCA GAT CAT ATC AAT TTT GAT CCT GTA TAT AAA GTG AAG CCC AAT 912
Thr Ser Asp His Ile Asn Phe Asp Pro Val Tyr Lys Val Lys Pro Asn
190 195 200

CCG CCA CAT AAT TTA TCA GTG ATC AAC TCA GAG GAA CTG TCT AGT ATC 960
Pro Pro His Asn Leu Ser Val Ile Asn Ser Glu Glu Leu Ser Ser Ile
205 210 215

TTA AAA TTG ACA TGG ACC AAC CCA AGT ATT AAG AGT GTT ATA ATA CTA 1008
Leu Lys Leu Thr Trp Thr Asn Pro Ser Ile Lys Ser Val Ile Ile Leu
220 225 230

AAA TAT AAC ATT CAA TAT AGG ACC AAA GAT GCC TCA ACT TGG AGC CAG 1056
Lys Tyr Asn Ile Gln Tyr Arg Thr Lys Asp Ala Ser Thr Trp Ser Gln
235 240 245

ATT CCT CCT GAA GAC ACA GCA TCC ACC CGA TCT TCA TTC ACT GTC CAA 1104
Ile Pro Pro Glu Asp Thr Ala Ser Thr Arg Ser Ser Phe Thr Val Gln
250 255 260 265

GAC CTT AAA CCT TTT ACA GAA TAT GTG TTT AGG ATT CGC TGT ATG AAG 1152
Asp Leu Lys Pro Phe Thr Glu Tyr Val Phe Arg Ile Arg Cys Met Lys
270 275 280

GAA GAT GGT AAG GGA TAC TGG AGT GAC TGG AGT GAA GAA GCA AGT GGG 1200
Glu Asp Gly Lys Gly Tyr Trp Ser Asp Trp Ser Glu Glu Ala Ser Gly
285 290 295

ATC ACC TAT GAA GAT AGA CCA TCT AAA GCA CCA AGT TTC TGG TAT AAA 1248
Ile Thr Tyr Glu Asp Arg Pro Ser Lys Ala Pro Ser Phe Trp Tyr Lys
300 305 310

ATA GAT CCA TCC CAT ACT CAA GGC TAC AGA ACT GTA CAA CTC GTG TGG 1296
Ile Asp Pro Ser His Thr Gln Gly Tyr Arg Thr Val Gln Leu Val Trp
315 320 325

AAG ACA TTG CCT CCT TTT GAA GCC AAT GGA AAA ATC TTG GAT TAT GAA 1344
Lys Thr Leu Pro Pro Phe Glu Ala Asn Gly Lys Ile Leu Asp Tyr Glu
330 335 340 345

GTG ACT CTC ACA AGA TGG AAA TCA CAT TTA CAA AAT TAC ACA GTT AAT 1392
Val Thr Leu Thr Arg Trp Lys Ser His Leu Gln Asn Tyr Thr Val Asn
350 355 360

GCC ACA AAA CTG ACA GTA AAT CTC ACA AAT GAT CGC TAT CTA GCA ACC 1440
Ala Thr Lys Leu Thr Val Asn Leu Thr Asn Asp Arg Tyr Leu Ala Thr
365 370 375

CTA ACA GTA AGA AAT CTT GTT GGC AAA TCA GAT GCA GCT GTT TTA ACT 1488
Leu Thr Val Arg Asn Leu Val Gly Lys Ser Asp Ala Ala Val Leu Thr
380 385 390

ATC CCT GCC TGT GAC TTT CAA GCT ACT CAC CCT GTA ATG GAT CTT AAA 1536
Ile Pro Ala Cys Asp Phe Gln Ala Thr His Pro Val Met Asp Leu Lys
395 400 405

GCA TTC CCC AAA GAT AAC ATG CTT TGG GTG GAA TGG ACT ACT CCA AGG 1584
Ala Phe Pro Lys Asp Asn Met Leu Trp Val Glu Trp Thr Thr Pro Arg
410 415 420 425

GAA TCT GTA AAG AAA TAT ATA CTT GAG TGG TGT GTG TTA TCA GAT AAA 1632
Glu Ser Val Lys Lys Tyr Ile Leu Glu Trp Cys Val Leu Ser Asp Lys
430 435 440

GCA CCC TGT ATC ACA GAC TGG CAA CAA GAA GAT GGT ACC GTG CAT CGC 1680
Ala Pro Cys Ile Thr Asp Trp Gln Gln Glu Asp Gly Thr Val His Arg
445 450 455

ACC TAT TTA AGA GGG AAC TTA GCA GAG AGC AAA TGC TAT TTG ATA ACA 1728
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460 465 470

GTT ACT CCA GTA TAT GCT GAT GGA CCA GGA AGC CCT GAA TCC ATA AAG 1776
Val Thr Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro Glu Ser Ile Lys
475 480 485

GCA TAC CTT AAA CAA GCT CCA CCT TCC AAA GGA CCT ACT GTT CGG ACA 1824
Ala Tyr Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro Thr Val Arg Thr
490 495 500 505

AAA AAA GTA GGG AAA AAC GAA GCT GTC TTA GAG TGG GAC CAA CTT CCT 1872
Lys Lys Val Gly Lys Asn Glu Ala Val Leu Glu Trp Asp Gln Leu Pro
510 515 520

GTT GAT GTT CAG AAT GGA TTT ATC AGA AAT TAT ACT ATA TTT TAT AGA 1920
Val Asp Val Gln Asn Gly Phe Ile Arg Asn Tyr Thr Ile Phe Tyr Arg
525 530 535

ACC ATC ATT GGA AAT GAA ACT GCT GTG AAT GTG GAT TCT TCC CAC ACA 1968
Thr Ile Ile Gly Asn Glu Thr Ala Val Asn Val Asp Ser Ser His Thr
540 545 550

GAA TAT ACA TTG TCC TCT TTG ACT AGT GAC ACA TTG TAC ATG GTA CGA 2016
Glu Tyr Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu Tyr Met Val Arg
555 560 565

ATG GCA GCA TAC ACA GAT GAA GGT GGG AAG GAT GGT CCA GAA TTC ACT 2064
Met Ala Ala Tyr Thr Asp Glu Gly Gly Lys Asp Gly Pro Glu Phe Thr
570 575 580 585

TTT ACT ACC CCA AAG TTT GCT CAA GGA GAA ATT GAA GCC ATA GTC GTG 2112
Phe Thr Thr Pro Lys Phe Ala Gln Gly Glu Ile Glu Ala Ile Val Val
590 595 600

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CCT GTT TGC TTA GCA TTC CTA TTG ACA ACT CTT CTG GGA GTG CTG TTC     2160
Pro Val Cys Leu Ala Phe Leu Leu Thr Thr Leu Leu Gly Val Leu Phe
            605                 610                 615

TGC TTT AAT AAG CGA GAC CTA ATT AAA AAA CAC ATC TGG CCT AAT GTT     2208
Cys Phe Asn Lys Arg Asp Leu Ile Lys Lys His Ile Trp Pro Asn Val
        620                 625                 630

CCA GAT CCT TCA AAG AGT CAT ATT GCC CAG TGG TCA CCT CAC ACT CCT     2256
Pro Asp Pro Ser Lys Ser His Ile Ala Gln Trp Ser Pro His Thr Pro
    635                 640                 645

CCA AGG CAC AAT TTT AAT TCA AAA GAT CAA ATG TAT TCA GAT GGC AAT     2304
Pro Arg His Asn Phe Asn Ser Lys Asp Gln Met Tyr Ser Asp Gly Asn
650                 655                 660                 665

TTC ACT GAT GTA AGT GTT GTG GAA ATA GAA GCA AAT GAC AAA AAG CCT     2352
Phe Thr Asp Val Ser Val Val Glu Ile Glu Ala Asn Asp Lys Lys Pro
                670                 675                 680

TTT CCA GAA GAT CTG AA                                              2369
Phe Pro Glu Asp Leu
            685
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 708 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Thr Thr Glu Ser Thr Gly Glu Leu Leu Asp Pro Cys Gly Tyr Ile Ser
     -5                  1               5                  10

Pro Glu Ser Pro Val Val Gln Leu His Ser Asn Phe Thr Ala Val Cys
                15                  20                  25

Val Leu Lys Glu Lys Cys Met Asp Tyr Phe His Val Asn Ala Asn Tyr
            30                  35                  40

Ile Val Trp Lys Thr Asn His Phe Thr Ile Pro Lys Glu Gln Tyr Thr
        45                  50                  55

Ile Ile Asn Arg Thr Ala Ser Ser Val Thr Phe Thr Asp Ile Ala Ser
    60                  65                  70

Leu Asn Ile Gln Leu Thr Cys Asn Ile Leu Thr Phe Gly Gln Leu Glu
75                  80                  85                  90

Gln Asn Val Tyr Gly Ile Thr Ile Ile Ser Gly Leu Pro Pro Glu Lys
                95                 100                 105
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Pro Lys Asn Leu Ser Cys Ile Val Asn Glu Gly Lys Lys Met Arg Cys
110 115 120

Glu Trp Asp Gly Gly Arg Glu Thr His Leu Glu Thr Asn Phe Thr Leu
125 130 135

Lys Ser Glu Trp Ala Thr His Lys Phe Ala Asp Cys Lys Ala Lys Arg
140 145 150

Asp Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser Thr Val Tyr Phe Val
155 160 165 170

Asn Ile Glu Val Trp Val Glu Ala Glu Asn Ala Leu Gly Lys Val Thr
175 180 185

Ser Asp His Ile Asn Phe Asp Pro Val Tyr Lys Val Lys Pro Asn Pro
190 195 200

Pro His Asn Leu Ser Val Ile Asn Ser Glu Glu Leu Ser Ser Ile Leu
205 210 215

Lys Leu Thr Trp Thr Asn Pro Ser Ile Lys Ser Val Ile Ile Leu Lys
220 225 230

Tyr Asn Ile Gln Tyr Arg Thr Lys Asp Ala Ser Thr Trp Ser Gln Ile
235 240 245 250

Pro Pro Glu Asp Thr Ala Ser Thr Arg Ser Ser Phe Thr Val Gln Asp
255 260 265

Leu Lys Pro Phe Thr Glu Tyr Val Phe Arg Ile Arg Cys Met Lys Glu
270 275 280

Asp Gly Lys Gly Tyr Trp Ser Asp Trp Ser Glu Glu Ala Ser Gly Ile
285 290 295

Thr Tyr Glu Asp Arg Pro Ser Lys Ala Pro Ser Phe Trp Tyr Lys Ile
300 305 310

Asp Pro Ser His Thr Gln Gly Tyr Arg Thr Val Gln Leu Val Trp Lys
315 320 325 330

Thr Leu Pro Pro Phe Glu Ala Asn Gly Lys Ile Leu Asp Tyr Glu Val
335 340 345

Thr Leu Thr Arg Trp Lys Ser His Leu Gln Asn Tyr Thr Val Asn Ala
350 355 360

Thr Lys Leu Thr Val Asn Leu Thr Asn Asp Arg Tyr Leu Ala Thr Leu
365 370 375

Thr Val Arg Asn Leu Val Gly Lys Ser Asp Ala Ala Val Leu Thr Ile
380 385 390

Pro Ala Cys Asp Phe Gln Ala Thr His Pro Val Met Asp Leu Lys Ala
395 400 405 410

Phe Pro Lys Asp Asn Met Leu Trp Val Glu Trp Thr Thr Pro Arg Glu
415 420 425

Ser Val Lys Lys Tyr Ile Leu Glu Trp Cys Val Leu Ser Asp Lys Ala
430 435 440

Pro Cys Ile Thr Asp Trp Gln Gln Glu Asp Gly Thr Val His Arg Thr
445 450 455

Tyr Leu Arg Gly Asn Leu Ala Glu Ser Lys Cys Tyr Leu Ile Thr Val
460 465 470

Thr Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro Glu Ser Ile Lys Ala
475 480 485 490

Tyr Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro Thr Val Arg Thr Lys
495 500 505

Lys Val Gly Lys Asn Glu Ala Val Leu Glu Trp Asp Gln Leu Pro Val
510 515 520

Asp Val Gln Asn Gly Phe Ile Arg Asn Tyr Thr Ile Phe Tyr Arg Thr
525 530 535

Ile Ile Gly Asn Glu Thr Ala Val Asn Val Asp Ser Ser His Thr Glu
540 545 550

Tyr Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu Tyr Met Val Arg Met
555 560 565 570

Ala Ala Tyr Thr Asp Glu Gly Gly Lys Asp Gly Pro Glu Phe Thr Phe
575 580 585

Thr Thr Pro Lys Phe Ala Gln Gly Glu Ile Glu Ala Ile Val Val Pro
590 595 600

Val Cys Leu Ala Phe Leu Leu Thr Thr Leu Leu Gly Val Leu Phe Cys
605 610 615

Phe Asn Lys Arg Asp Leu Ile Lys Lys His Ile Trp Pro Asn Val Pro
620 625 630

Asp Pro Ser Lys Ser His Ile Ala Gln Trp Ser Pro His Thr Pro Pro
635 640 645 650

Arg His Asn Phe Asn Ser Lys Asp Gln Met Tyr Ser Asp Gly Asn Phe
655 660 665

Thr Asp Val Ser Val Val Glu Ile Glu Ala Asn Asp Lys Lys Pro Phe
670 675 680

Pro Glu Asp Leu
685

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 705 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: hIgG1Fc

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..699

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GAG CCC AGA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA      48
Glu Pro Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
  1               5                  10                  15

CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC      96
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
             20                  25                  30

AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG     144
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
         35                  40                  45

GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG     192
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
     50                  55                  60

GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG     240
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
 65                  70                  75                  80

TAC AAC AGC ACG TAC CGG GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG     288
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                 85                  90                  95

GAC TGG CTG AAT GGC AAG GAC TAC AAG TGC AAG GTC TCC AAC AAA GCC     336
Asp Trp Leu Asn Gly Lys Asp Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110

CTC CCA GCC CCC ATG CAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC     384
Leu Pro Ala Pro Met Gln Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        115                 120                 125

CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC     432
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
    130                 135                 140

AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGG     480
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Arg
145                 150                 155                 160

CAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC     528
His Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175
```

```
AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC        576
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190

AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC        624
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205

TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG        672
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220

AGC CTC TCC CTG TCT CCG GGT AAA TGAACTAGT                              705
Ser Leu Ser Leu Ser Pro Gly Lys
225                     230
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Glu Pro Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1               5                   10                  15

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        35                  40                  45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    50                  55                  60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65                  70                  75                  80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95

Asp Trp Leu Asn Gly Lys Asp Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110

Leu Pro Ala Pro Met Gln Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        115                 120                 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
    130                 135                 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Arg
145                 150                 155                 160

His Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175
```

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220

Ser Leu Ser Leu Ser Pro Gly Lys
225 230

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3182 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: human placenta

(vii) IMMEDIATE SOURCE:
(B) CLONE: pHLIFR-65

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 311..3182

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 179..3182

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 179..310

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 311..3182

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGATCTTGGA ACGAGACGAC CTGCTCTCTC TCCCAGAACG TGTCTCTGCT GCAAGGCACC 60

GGGCCCTTTC GCTCTGCAGA ACTGCACTTG CAAGACCATT ATCAACTCCT AATCCCAGCT 120

CAGAAAGGGA GCCTCTGCGA CTCATTCATC GCCCTCCAGG ACTGACTGCA TTGCACAG 178

```
ATG ATG GAT ATT TAC GTA TGT TTG AAA CGA CCA TCC TGG ATG GTG GAC    226
Met Met Asp Ile Tyr Val Cys Leu Lys Arg Pro Ser Trp Met Val Asp
-44             -40                 -35                 -30

AAT AAA AGA ATG AGG ACT GCT TCA AAT TTC CAG TGG CTG TTA TCA ACA    274
Asn Lys Arg Met Arg Thr Ala Ser Asn Phe Gln Trp Leu Leu Ser Thr
            -25                 -20                 -15

TTT ATT CTT CTA TAT CTA ATG AAT CAA GTA AAT AGC CAG AAA AAG GGG    322
Phe Ile Leu Leu Tyr Leu Met Asn Gln Val Asn Ser Gln Lys Lys Gly
        -10                 -5              1

GCT CCT CAT GAT TTG AAG TGT GTA ACT AAC AAT TTG CAA GTG TGG AAC    370
Ala Pro His Asp Leu Lys Cys Val Thr Asn Asn Leu Gln Val Trp Asn
5                   10                  15                  20

TGT TCT TGG AAA GCA CCC TCT GGA ACA GGC CGT GGT ACT GAT TAT GAA    418
Cys Ser Trp Lys Ala Pro Ser Gly Thr Gly Arg Gly Thr Asp Tyr Glu
                25                  30                  35

GTT TGC ATT GAA AAC AGG TCC CGT TCT TGT TAT CAG TTG GAG AAA ACC    466
Val Cys Ile Glu Asn Arg Ser Arg Ser Cys Tyr Gln Leu Glu Lys Thr
            40                  45                  50

AGT ATT AAA ATT CCA GCT CTT TCA CAT GGT GAT TAT GAA ATA ACA ATA    514
Ser Ile Lys Ile Pro Ala Leu Ser His Gly Asp Tyr Glu Ile Thr Ile
        55                  60                  65

AAT TCT CTA CAT GAT TTT GGA AGT TCT ACA AGT AAA TTC ACA CTA AAT    562
Asn Ser Leu His Asp Phe Gly Ser Ser Thr Ser Lys Phe Thr Leu Asn
    70                  75                  80

GAA CAA AAC GTT TCC TTA ATT CCA GAT ACT CCA GAG ATC TTG AAT TTG    610
Glu Gln Asn Val Ser Leu Ile Pro Asp Thr Pro Glu Ile Leu Asn Leu
85                  90                  95                  100

TCT GCT GAT TTC TCA ACC TCT ACA TTA TAC CTA AAG TGG AAC GAC AGG    658
Ser Ala Asp Phe Ser Thr Ser Thr Leu Tyr Leu Lys Trp Asn Asp Arg
                105                 110                 115

GGT TCA GTT TTT CCA CAC CGC TCA AAT GTT ATC TGG GAA ATT AAA GTT    706
Gly Ser Val Phe Pro His Arg Ser Asn Val Ile Trp Glu Ile Lys Val
            120                 125                 130

CTA CGT AAA GAG AGT ATG GAG CTC GTA AAA TTA GTG ACC CAC AAC ACA    754
Leu Arg Lys Glu Ser Met Glu Leu Val Lys Leu Val Thr His Asn Thr
        135                 140                 145

ACT CTG AAT GGC AAA GAT ACA CTT CAT CAC TGG AGT TGG GCC TCA GAT    802
Thr Leu Asn Gly Lys Asp Thr Leu His His Trp Ser Trp Ala Ser Asp
    150                 155                 160

ATG CCC TTG GAA TGT GCC ATT CAT TTT GTG GAA ATT AGA TGC TAC ATT    850
Met Pro Leu Glu Cys Ala Ile His Phe Val Glu Ile Arg Cys Tyr Ile
165                 170                 175                 180

GAC AAT CTT CAT TTT TCT GGT CTC GAA GAG TGG AGT GAC TGG AGC CCT    898
Asp Asn Leu His Phe Ser Gly Leu Glu Glu Trp Ser Asp Trp Ser Pro
                185                 190                 195
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GTG AAG AAC ATT TCT TGG ATA CCT GAT TCT CAG ACT AAG GTT TTT CCT 946
Val Lys Asn Ile Ser Trp Ile Pro Asp Ser Gln Thr Lys Val Phe Pro
200 205 210

CAA GAT AAA GTG ATA CTT GTA GGC TCA GAC ATA ACA TTT TGT TGT GTG 994
Gln Asp Lys Val Ile Leu Val Gly Ser Asp Ile Thr Phe Cys Cys Val
215 220 225

AGT CAA GAA AAA GTG TTA TCA GCA CTG ATT GGC CAT ACA AAC TGC CCC 1042
Ser Gln Glu Lys Val Leu Ser Ala Leu Ile Gly His Thr Asn Cys Pro
230 235 240

TTG ATC CAT CTT GAT GGG GAA AAT GTT GCA ATC AAG ATT CGT AAT ATT 1090
Leu Ile His Leu Asp Gly Glu Asn Val Ala Ile Lys Ile Arg Asn Ile
245 250 255 260

TCT GTT TCT GCA AGT AGT GGA ACA AAT GTA GTT TTT ACA ACC GAA GAT 1138
Ser Val Ser Ala Ser Ser Gly Thr Asn Val Val Phe Thr Thr Glu Asp
265 270 275

AAC ATA TTT GGA ACC GTT ATT TTT GCT GGA TAT CCA CCA GAT ACT CCT 1186
Asn Ile Phe Gly Thr Val Ile Phe Ala Gly Tyr Pro Pro Asp Thr Pro
280 285 290

CAA CAA CTG AAT TGT GAG ACA CAT GAT TTA AAA GAA ATT ATA TGT AGT 1234
Gln Gln Leu Asn Cys Glu Thr His Asp Leu Lys Glu Ile Ile Cys Ser
295 300 305

TGG AAT CCA GGA AGG GTG ACA GCG TTG GTG GGC CCA CGT GCT ACA AGC 1282
Trp Asn Pro Gly Arg Val Thr Ala Leu Val Gly Pro Arg Ala Thr Ser
310 315 320

TAC ACT TTA GTT GAA AGT TTT TCA GGA AAA TAT GTT AGA CTT AAA AGA 1330
Tyr Thr Leu Val Glu Ser Phe Ser Gly Lys Tyr Val Arg Leu Lys Arg
325 330 335 340

GCT GAA GCA CCT ACA AAC GAA AGC TAT CAA TTA TTA TTT CAA ATG CTT 1378
Ala Glu Ala Pro Thr Asn Glu Ser Tyr Gln Leu Leu Phe Gln Met Leu
345 350 355

CCA AAT CAA GAA ATA TAT AAT TTT ACT TTG AAT GCT CAC AAT CCG CTG 1426
Pro Asn Gln Glu Ile Tyr Asn Phe Thr Leu Asn Ala His Asn Pro Leu
360 365 370

GGT CGA TCA CAA TCA ACA ATT TTA GTT AAT ATA ACT GAA AAA GTT TAT 1474
Gly Arg Ser Gln Ser Thr Ile Leu Val Asn Ile Thr Glu Lys Val Tyr
375 380 385

CCC CAT ACT CCT ACT TCA TTC AAA GTG AAG GAT ATT AAT TCA ACA GCT 1522
Pro His Thr Pro Thr Ser Phe Lys Val Lys Asp Ile Asn Ser Thr Ala
390 395 400

GTT AAA CTT TCT TGG CAT TTA CCA GGC AAC TTT GCA AAG ATT AAT TTT 1570
Val Lys Leu Ser Trp His Leu Pro Gly Asn Phe Ala Lys Ile Asn Phe
405 410 415 420

TTA TGT GAA ATT GAA ATT AAG AAA TCT AAT TCA GTA CAA GAG CAG CGG 1618
Leu Cys Glu Ile Glu Ile Lys Lys Ser Asn Ser Val Gln Glu Gln Arg
425 430 435

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AAT GTC ACA ATC AAA GGA GTA GAA AAT TCA AGT TAT CTT GTT GCT CTG    1666
Asn Val Thr Ile Lys Gly Val Glu Asn Ser Ser Tyr Leu Val Ala Leu
        440                     445                 450

GAC AAG TTA AAT CCA TAC ACT CTA TAT ACT TTT CGG ATT CGT TGT TCT    1714
Asp Lys Leu Asn Pro Tyr Thr Leu Tyr Thr Phe Arg Ile Arg Cys Ser
    455                     460                 465

ACT GAA ACT TTC TGG AAA TGG AGC AAA TGG AGC AAT AAA AAA CAA CAT    1762
Thr Glu Thr Phe Trp Lys Trp Ser Lys Trp Ser Asn Lys Lys Gln His
    470                 475                     480

TTA ACA ACA GAA GCC AGT CCT TCA AAG GGG CCT GAT ACT TGG AGA GAG    1810
Leu Thr Thr Glu Ala Ser Pro Ser Lys Gly Pro Asp Thr Trp Arg Glu
485                     490                 495                 500

TGG AGT TCT GAT GGA AAA AAT TTA ATA ATC TAT TGG AAG CCT TTA CCC    1858
Trp Ser Ser Asp Gly Lys Asn Leu Ile Ile Tyr Trp Lys Pro Leu Pro
                505                     510                 515

ATT AAT GAA GCT AAT GGA AAA ATA CTT TCC TAC AAT GTA TCG TGT TCA    1906
Ile Asn Glu Ala Asn Gly Lys Ile Leu Ser Tyr Asn Val Ser Cys Ser
            520                     525                 530

TCA GAT GAG GAA ACA CAG TCC CTT TCT GAA ATC CCT GAT CCT CAG CAC    1954
Ser Asp Glu Glu Thr Gln Ser Leu Ser Glu Ile Pro Asp Pro Gln His
        535                     540                 545

AAA GCA GAG ATA CGA CTT GAT AAG AAT GAC TAC ATC ATC AGC GTA GTG    2002
Lys Ala Glu Ile Arg Leu Asp Lys Asn Asp Tyr Ile Ile Ser Val Val
    550                     555                 560

GCT AAA AAT TCT GTG GGC TCA TCA CCA CCT TCC AAA ATA GCG AGT ATG    2050
Ala Lys Asn Ser Val Gly Ser Ser Pro Pro Ser Lys Ile Ala Ser Met
565                     570                 575                 580

GAA ATT CCA AAT GAT GAT CTC AAA ATA GAA CAA GTT GTT GGG ATG GGA    2098
Glu Ile Pro Asn Asp Asp Leu Lys Ile Glu Gln Val Val Gly Met Gly
                585                     590                 595

AAG GGG ATT CTC CTC ACC TGG CAT TAC GAC CCC AAC ATG ACT TGC GAC    2146
Lys Gly Ile Leu Leu Thr Trp His Tyr Asp Pro Asn Met Thr Cys Asp
            600                     605                 610

TAC GTC ATT AAG TGG TGT AAC TCG TCT CGG TCG GAA CCA TGC CTT ATG    2194
Tyr Val Ile Lys Trp Cys Asn Ser Ser Arg Ser Glu Pro Cys Leu Met
        615                     620                 625

GAC TGG AGA AAA GTT CCC TCA AAC AGC ACT GAA ACT GTA ATA GAA TCT    2242
Asp Trp Arg Lys Val Pro Ser Asn Ser Thr Glu Thr Val Ile Glu Ser
    630                     635                 640

GAT GAG TTT CGA CCA GGT ATA AGA TAT AAT TTT TTC CTG TAT GGA TGC    2290
Asp Glu Phe Arg Pro Gly Ile Arg Tyr Asn Phe Phe Leu Tyr Gly Cys
645                     650                 655                 660

AGA AAT CAA GGA TAT CAA TTA TTA CGC TCC ATG ATT GGA TAT ATA GAA    2338
Arg Asn Gln Gly Tyr Gln Leu Leu Arg Ser Met Ile Gly Tyr Ile Glu
                665                     670                 675
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GAA TTG GCT CCC ATT GTT GCA CCA AAT TTT ACT GTT GAG GAT ACT TCT 2386
Glu Leu Ala Pro Ile Val Ala Pro Asn Phe Thr Val Glu Asp Thr Ser
680 685 690

GCA GAT TCG ATA TTA GTA AAA TGG GAA GAC ATT CCT GTG GAA GAA CTT 2434
Ala Asp Ser Ile Leu Val Lys Trp Glu Asp Ile Pro Val Glu Glu Leu
695 700 705

AGA GGC TTT TTA AGA GGA TAT TTG TTT TAC TTT GGA AAA GGA GAA AGA 2482
Arg Gly Phe Leu Arg Gly Tyr Leu Phe Tyr Phe Gly Lys Gly Glu Arg
710 715 720

GAC ACA TCT AAG ATG AGG GTT TTA GAA TCA GGT CGT TCT GAC ATA AAA 2530
Asp Thr Ser Lys Met Arg Val Leu Glu Ser Gly Arg Ser Asp Ile Lys
725 730 735 740

GTT AAG AAT ATT ACT GAC ATA TCC CAG AAG ACA CTG AGA ATT GCT GAT 2578
Val Lys Asn Ile Thr Asp Ile Ser Gln Lys Thr Leu Arg Ile Ala Asp
745 750 755

CTT CAA GGT AAA ACA AGT TAC CAC CTG GTC TTG CGA GCC TAT ACA GAT 2626
Leu Gln Gly Lys Thr Ser Tyr His Leu Val Leu Arg Ala Tyr Thr Asp
760 765 770

GGT GGA GTG GGC CCG GAG AAG AGT ATG TAT GTG GTG ACA AAG GAA AAT 2674
Gly Gly Val Gly Pro Glu Lys Ser Met Tyr Val Val Thr Lys Glu Asn
775 780 785

TCT GTG GGA TTA ATT ATT GCC ATT CTC ATC CCA GTG GCA GTG GCT GTC 2722
Ser Val Gly Leu Ile Ile Ala Ile Leu Ile Pro Val Ala Val Ala Val
790 795 800

ATT GTT GGA GTG GTG ACA AGT ATC CTT TGC TAT CGG AAA CGA GAA TGG 2770
Ile Val Gly Val Val Thr Ser Ile Leu Cys Tyr Arg Lys Arg Glu Trp
805 810 815 820

ATT AAA GAA ACC TTC TAC CCT GAT ATT CCA AAT CCA GAA AAC TGT AAA 2818
Ile Lys Glu Thr Phe Tyr Pro Asp Ile Pro Asn Pro Glu Asn Cys Lys
825 830 835

GCA TTA CAG TTT CAA AAG AGT GTC TGT GAG GGA AGC AGT GCT CTT AAA 2866
Ala Leu Gln Phe Gln Lys Ser Val Cys Glu Gly Ser Ser Ala Leu Lys
840 845 850

ACA TTG GAA ATG AAT CCT TGT ACC CCA AAT AAT GTT GAG GTT CTG GAA 2914
Thr Leu Glu Met Asn Pro Cys Thr Pro Asn Asn Val Glu Val Leu Glu
855 860 865

ACT CGA TCA GCA TTT CCT AAA ATA GAA GAT ACA GAA ATA ATT TCC CCA 2962
Thr Arg Ser Ala Phe Pro Lys Ile Glu Asp Thr Glu Ile Ile Ser Pro
870 875 880

GTA GCT GAG CGT CCT GAA GAT CGC TCT GAT GCA GAG CCT GAA AAC CAT 3010
Val Ala Glu Arg Pro Glu Asp Arg Ser Asp Ala Glu Pro Glu Asn His
885 890 895 900

GTG GTT GTG TCC TAT TGT CCA CCC ATC ATT GAG GAA GAA ATA CCA AAC 3058
Val Val Val Ser Tyr Cys Pro Pro Ile Ile Glu Glu Glu Ile Pro Asn
905 910 915

```
CCA GCC GCA GAT GAA GCT GGA GGG ACT GCA CAG GTT ATT TAC ATT GAT      3106
Pro Ala Ala Asp Glu Ala Gly Gly Thr Ala Gln Val Ile Tyr Ile Asp
            920                 925                 930

GTT CAG TCG ATG TAT CAG CCT CAA GCA AAA CCA GAA GAA AAA AAA AAA      3154
Val Gln Ser Met Tyr Gln Pro Gln Ala Lys Pro Glu Glu Lys Lys Lys
        935                 940                 945

AAA AGC AGG TCG TCT CGT TCC AAG ATC T                                3182
Lys Ser Arg Ser Ser Arg Ser Lys Ile
    950                 955
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1001 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Met Asp Ile Tyr Val Cys Leu Lys Arg Pro Ser Trp Met Val Asp
-44             -40                 -35                 -30

Asn Lys Arg Met Arg Thr Ala Ser Asn Phe Gln Trp Leu Leu Ser Thr
            -25                 -20                 -15

Phe Ile Leu Leu Tyr Leu Met Asn Gln Val Asn Ser Gln Lys Lys Gly
        -10                 -5                   1

Ala Pro His Asp Leu Lys Cys Val Thr Asn Asn Leu Gln Val Trp Asn
  5                  10                  15                  20

Cys Ser Trp Lys Ala Pro Ser Gly Thr Gly Arg Gly Thr Asp Tyr Glu
                 25                  30                  35

Val Cys Ile Glu Asn Arg Ser Arg Ser Cys Tyr Gln Leu Glu Lys Thr
             40                  45                  50

Ser Ile Lys Ile Pro Ala Leu Ser His Gly Asp Tyr Glu Ile Thr Ile
         55                  60                  65

Asn Ser Leu His Asp Phe Gly Ser Ser Thr Ser Lys Phe Thr Leu Asn
     70                  75                  80

Glu Gln Asn Val Ser Leu Ile Pro Asp Thr Pro Glu Ile Leu Asn Leu
 85                  90                  95                 100

Ser Ala Asp Phe Ser Thr Ser Thr Leu Tyr Leu Lys Trp Asn Asp Arg
                105                 110                 115

Gly Ser Val Phe Pro His Arg Ser Asn Val Ile Trp Glu Ile Lys Val
            120                 125                 130

Leu Arg Lys Glu Ser Met Glu Leu Val Lys Leu Val Thr His Asn Thr
        135                 140                 145
```

Thr Leu Asn Gly Lys Asp Thr Leu His His Trp Ser Trp Ala Ser Asp
150 155 160

Met Pro Leu Glu Cys Ala Ile His Phe Val Glu Ile Arg Cys Tyr Ile
165 170 175 180

Asp Asn Leu His Phe Ser Gly Leu Glu Glu Trp Ser Asp Trp Ser Pro
185 190 195

Val Lys Asn Ile Ser Trp Ile Pro Asp Ser Gln Thr Lys Val Phe Pro
200 205 210

Gln Asp Lys Val Ile Leu Val Gly Ser Asp Ile Thr Phe Cys Cys Val
215 220 225

Ser Gln Glu Lys Val Leu Ser Ala Leu Ile Gly His Thr Asn Cys Pro
230 235 240

Leu Ile His Leu Asp Gly Glu Asn Val Ala Ile Lys Ile Arg Asn Ile
245 250 255 260

Ser Val Ser Ala Ser Ser Gly Thr Asn Val Val Phe Thr Thr Glu Asp
265 270 275

Asn Ile Phe Gly Thr Val Ile Phe Ala Gly Tyr Pro Pro Asp Thr Pro
280 285 290

Gln Gln Leu Asn Cys Glu Thr His Asp Leu Lys Glu Ile Ile Cys Ser
295 300 305

Trp Asn Pro Gly Arg Val Thr Ala Leu Val Gly Pro Arg Ala Thr Ser
310 315 320

Tyr Thr Leu Val Gln Ser Phe Ser Gly Lys Tyr Val Arg Leu Lys Arg
325 330 335 340

Ala Glu Ala Pro Thr Asn Glu Ser Tyr Gln Leu Leu Phe Gln Met Leu
345 350 355

Pro Asn Gln Glu Ile Tyr Asn Phe Thr Leu Asn Ala His Asn Pro Leu
360 365 370

Gly Arg Ser Gln Ser Thr Ile Leu Val Asn Ile Thr Glu Lys Val Tyr
375 380 385

Pro His Thr Pro Thr Ser Phe Lys Val Lys Asp Ile Asn Ser Thr Ala
390 395 400

Val Lys Leu Ser Trp His Leu Pro Gly Asn Phe Ala Lys Ile Asn Phe
405 410 415 420

Leu Cys Glu Ile Glu Ile Lys Lys Ser Asn Ser Val Gln Glu Gln Arg
425 430 435

Asn Val Thr Ile Lys Gly Val Glu Asn Ser Ser Tyr Leu Val Ala Leu
440 445 450

Asp Lys Leu Asn Pro Tyr Thr Leu Tyr Thr Phe Arg Ile Arg Cys Ser
455 460 465

Thr Glu Thr Phe Trp Lys Trp Ser Lys Trp Ser Asn Lys Lys Gln His
470 475 480

Leu Thr Thr Glu Ala Ser Pro Ser Lys Gly Pro Asp Thr Trp Arg Glu
485 490 495 500

Trp Ser Ser Asp Gly Lys Asn Leu Ile Ile Tyr Trp Lys Pro Leu Pro
505 510 515

Ile Asn Glu Ala Asn Gly Lys Ile Leu Ser Tyr Asn Val Ser Cys Ser
520 525 530

Ser Asp Glu Glu Thr Gln Ser Leu Ser Glu Ile Pro Asp Pro Gln His
535 540 545

Lys Ala Glu Ile Arg Leu Asp Lys Asn Asp Tyr Ile Ile Ser Val Val
550 555 560

Ala Lys Asn Ser Val Gly Ser Ser Pro Pro Ser Lys Ile Ala Ser Met
565 570 575 580

Glu Ile Pro Asn Asp Asp Leu Lys Ile Glu Gln Val Val Gly Met Gly
585 590 595

Lys Gly Ile Leu Leu Thr Trp His Tyr Asp Pro Asn Met Thr Cys Asp
600 605 610

Tyr Val Ile Lys Trp Cys Asn Ser Ser Arg Ser Glu Pro Cys Leu Met
615 620 625

Asp Trp Arg Lys Val Pro Ser Asn Ser Thr Glu Thr Val Ile Glu Ser
630 635 640

Asp Glu Phe Arg Pro Gly Ile Arg Tyr Asn Phe Phe Leu Tyr Gly Cys
645 650 655 660

Arg Asn Gln Gly Tyr Gln Leu Leu Arg Ser Met Ile Gly Tyr Ile Glu
665 670 675

Glu Leu Ala Pro Ile Val Ala Pro Asn Phe Thr Val Glu Asp Thr Ser
680 685 690

Ala Asp Ser Ile Leu Val Lys Trp Glu Asp Ile Pro Val Glu Glu Leu
695 700 705

Arg Gly Phe Leu Arg Gly Tyr Leu Phe Tyr Phe Gly Lys Gly Glu Arg
710 715 720

Asp Thr Ser Lys Met Arg Val Leu Glu Ser Gly Arg Ser Asp Ile Lys
725 730 735 740

Val Lys Asn Ile Thr Asp Ile Ser Gln Lys Thr Leu Arg Ile Ala Asp
745 750 755

Leu Gln Gly Lys Thr Ser Tyr His Leu Val Leu Arg Ala Tyr Thr Asp
760 765 770

Gly Gly Val Gly Pro Glu Lys Ser Met Tyr Val Val Thr Lys Glu Asn
775 780 785

Ser Val Gly Leu Ile Ile Ala Ile Leu Ile Pro Val Ala Val Ala Val
790 795 800

Ile Val Gly Val Val Thr Ser Ile Leu Cys Tyr Arg Lys Arg Glu Trp
805 810 815 820

Ile Lys Glu Thr Phe Tyr Pro Asp Ile Pro Asn Pro Glu Asn Cys Lys
825 830 835

Ala Leu Gln Phe Gln Lys Ser Val Cys Glu Gly Ser Ser Ala Leu Lys
840 845 850

Thr Leu Glu Met Asn Pro Cys Thr Pro Asn Asn Val Glu Val Leu Glu
855 860 865

Thr Arg Ser Ala Phe Pro Lys Ile Glu Asp Thr Glu Ile Ile Ser Pro
870 875 880

Val Ala Glu Arg Pro Glu Asp Arg Ser Asp Ala Glu Pro Glu Asn His
885 890 895 900

Val Val Val Ser Tyr Cys Pro Pro Ile Ile Glu Glu Glu Ile Pro Asn
905 910 915

Pro Ala Ala Asp Glu Ala Gly Gly Thr Ala Gln Val Ile Tyr Ile Asp
920 925 930

Val Gln Ser Met Tyr Gln Pro Gln Ala Lys Pro Glu Glu Lys Lys Lys
935 940 945

Lys Ser Arg Ser Ser Arg Ser Lys Ile
950 955

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 100 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GATCCGGTGG AGGTGGTTCT GGTGGAGGTG GTTCAGGTGG TGGAGGATCA GGAGGTGGTG 60

GATCAGGTGG AGGAGGTTCT GGAGGTGGAG GTTCCGGAAT 100

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GATATGTCGA CGATGATGGA TATTTACGTA TGTTTG

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCATGGATCC ACCTCCTCCA GAATTTTCCT TTGTCACCAC ATACATAC

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCGTCCGGA GGAGGTGGAT CTGAACTTCT AGATCCATGT GGTTATATC

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCATGCGGCC GCCTATTCAA TTTCTCCTTG AGCAAAC

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GATATGTCGA CAAGATGTTG ACGTTGCAGA CTTGG

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCATGGATCC ACCTCCTCCT TCAATTTCTC CTTGAGCAAA C

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CGCGTCCGGA GGAGGTGGTA GCCAGAAAAA GGGGGCTCCT CATG

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GCATGCGGCC GCTAAGAATT TTCCTTTGTC ACCACATACA TAC

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCATAGATCT GGGCTCAGAA TTTTCCTTTG TCACCACATA CATAC

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GCATAGATCT GGGCTCTTCA ATTTCTCCTT GAGCAAAC

CLAIMS

What is claimed is:

1. A receptor capable of binding oncostatin M and leukemia inhibitory factor, comprising gp130 covalently linked to LIF-R.

2. A receptor according to claim 1, wherein said receptor comprises a soluble gp130 polypeptide covalently linked to a soluble LIF-R polypeptide.

3. A receptor according to claim 1 wherein said receptor comprises gp130 covalently linked to LIF-R *via* a polypeptide linker.

4. A receptor according to claim 3, wherein said receptor is a recombinant fusion protein of the formula:

$$R_1\text{-}L\text{-}R_2 \text{ or } R_2\text{-}L\text{-}R_1$$

wherein R_1 represents gp130; R_2 represents LIF-R; and L represents a polypeptide linker.

5. A receptor according to claim 4 wherein the polypeptide linker comprises from 20 to 100 amino acids selected from the group consisting of glycine, asparagine, serine, threonine, and alanine.

6. A receptor according to claim 5 wherein the polypeptide linker comprises an amino acid sequence selected from the group consisting of: $(Gly_4\text{-}Ser\text{-}Gly_5\text{-}Ser)_2$ and $(Gly_4\text{-}Ser)_n$, wherein n is 4-12.

7. An isolated DNA sequence encoding the receptor of claim 4.

8. A recombinant expression vector comprising the DNA sequence of claim 7.

9. A host cell containing the expression vector of claim 8.

10. A receptor according to claim 3, comprising a first fusion polypeptide that comprises an antibody Fc region polypeptide attached to the C-terminus of gp130, and a second fusion polypeptide that comprises an antibody Fc region polypeptide attached to the C-terminus of LIF-R, wherein said first fusion polypeptide is linked to said second fusion polypeptide *via* disulfide bonds between the Fc region polypeptides.

11. A receptor according to claim 1, 4, or 10, wherein:

a) said gp130 is encoded by an isolated DNA selected from the group consisting of a first DNA sequence comprising nucleotides 244-2369 of SEQ ID NO:1, a second DNA sequence comprising nucleotides 310-2369 of SEQ ID NO:1, and a third DNA sequence that will hybridize to said second DNA sequence under moderately stringent conditions; and

b) said LIF-R is encoded by an isolated DNA selected from the group consisting of a first DNA sequence comprising nucleotides 179-3182 of SEQ ID NO:5, a second DNA sequence comprising nucleotides 311-3182 of SEQ ID NO:5, and a third DNA sequence that will hybridize to said second DNA sequence under moderately stringent conditions.

12. A receptor according to claim 10 wherein said gp130 is a soluble gp130 polypeptide and said LIF-R is a soluble LIF-R polypeptide.

13. A fusion protein comprising an antibody Fc region polypeptide attached to the C-terminus of a soluble gp130 polypeptide.

14. An isolated DNA sequence encoding a fusion protein according to claim 13.

15. A fusion protein comprising an antibody Fc region polypeptide attached to the C-terminus of a soluble LIF-R polypeptide.

16. An isolated DNA sequence encoding a fusion protein according to claim 15.

17. A homodimeric receptor comprising two fusion proteins according to claim 15, linked via disulfide bonds between the Fc region polypeptides.

18. A process for preparing a receptor according to claim 4, comprising culturing a host cell transformed with an expression vector comprising a DNA sequence that encodes said fusion protein under conditions that promote expression of said fusion protein, and recovering said fusion protein.

19. A process for preparing a receptor according to claim 10, comprising culturing a host cell co-transfected with a first expression vector encoding said first fusion polypeptide and with a second expression vector encoding said second fusion polypeptide under conditions that promote expression of said first and second fusion polypeptides, and recovering said receptor.

20. A pharmaceutical composition for treating a disorder mediated by oncostatin M or LIF, comprising the receptor of claim 1, 4, 10, or 12, and a suitable diluent or carrier.

WO 93/10151 PCT/US92/10272

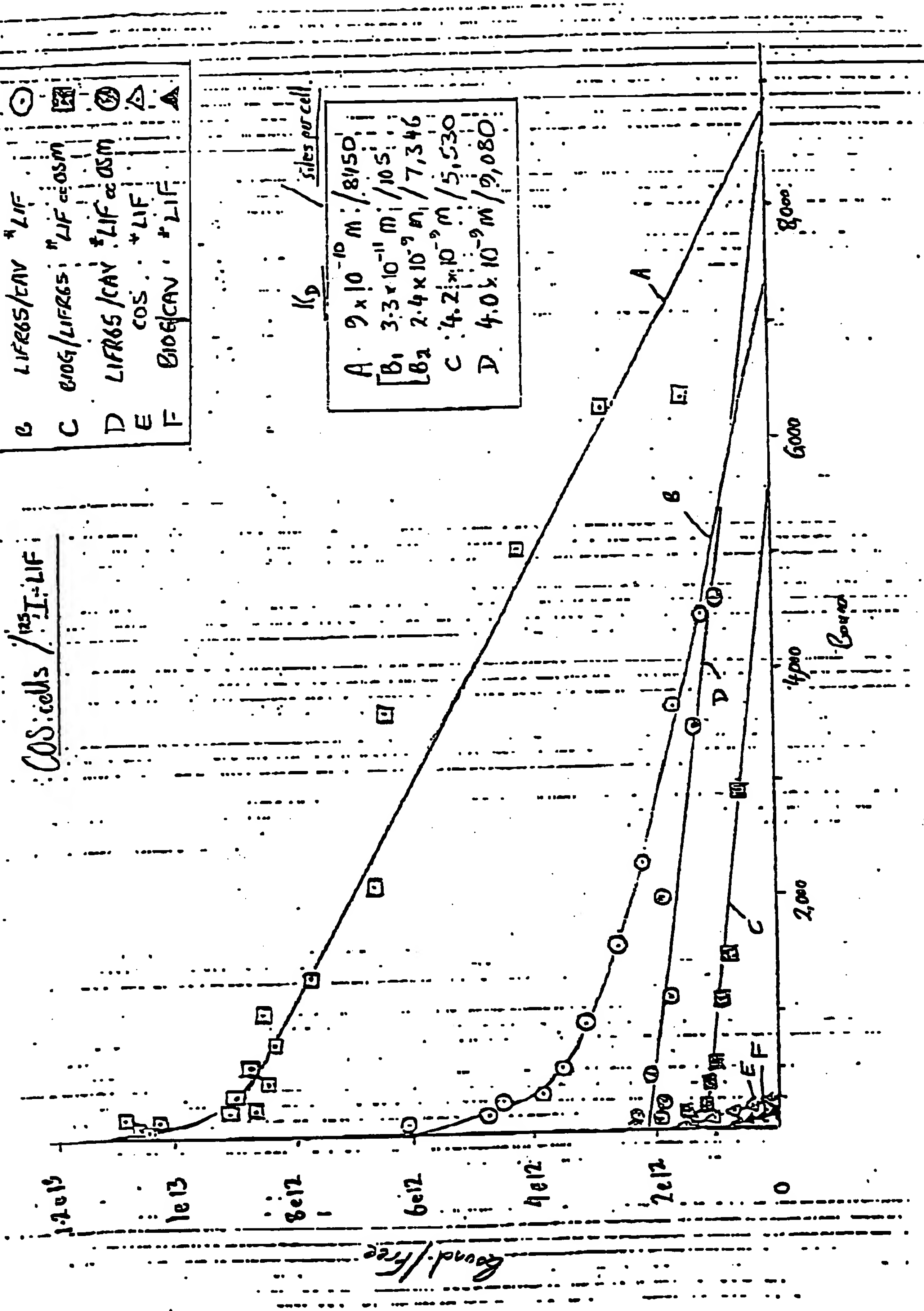


FIGURE 1

FIGURE 2

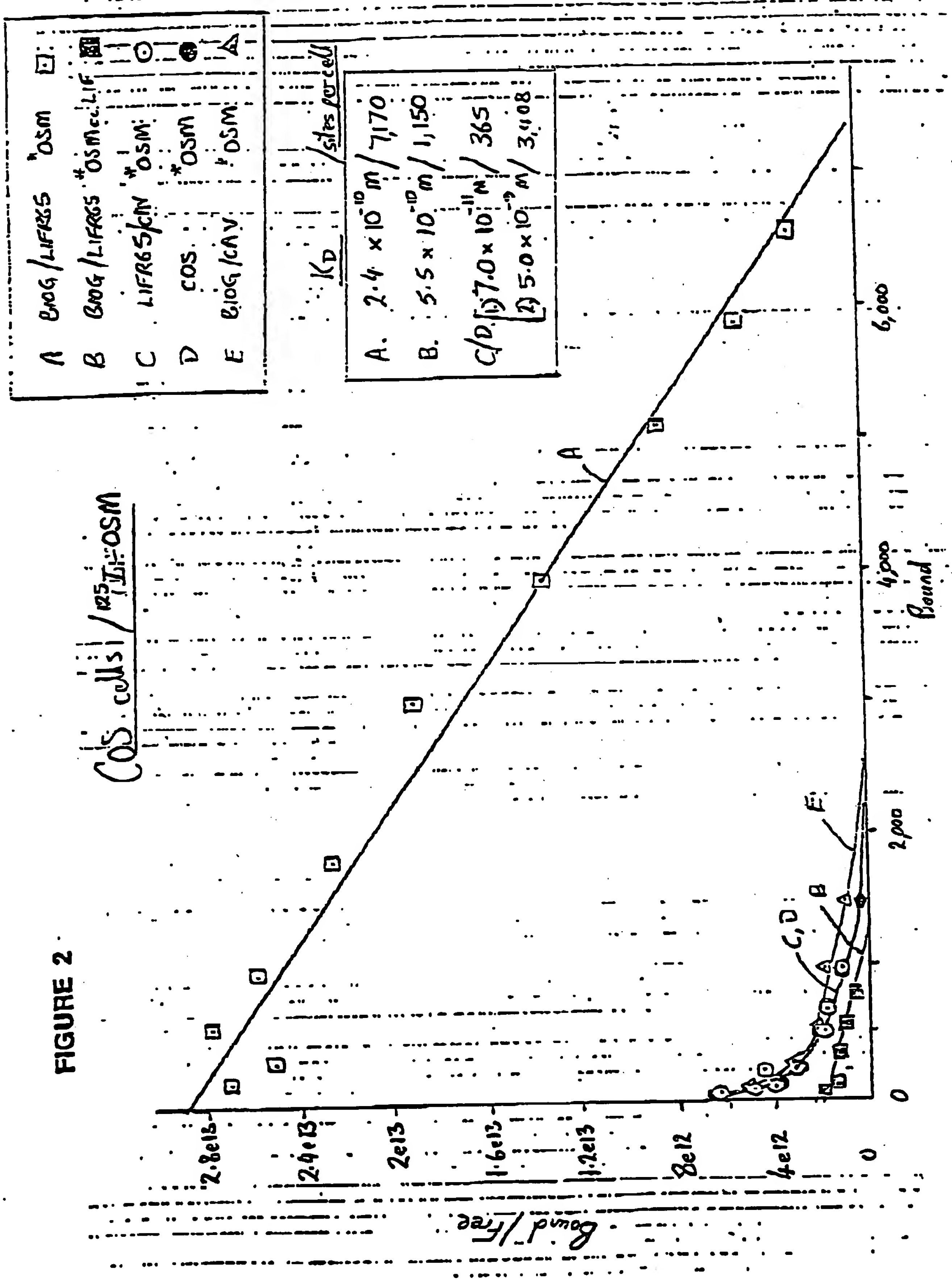

COS cells / 125I-LIF:OSM
A B10G/LIFR65 *OSM
B B10G/LIFR65 *OSM cc LIF
C LIFR65/CN *OSM
D COS *OSM
E B10G/CAV *OSM
KD / sites per cell
A. 2.4 x 10^-10 M / 7,170
B. 5.5 x 10^-10 M / 1,150
C/D (1) 7.0 x 10^-11 M / 365
(2) 5.0 x 10^-9 M / 3,408
A
B
C, D
E
Bound/Free
2.8e13
2.4e13
2e13
1.6e13
1.2e13
8e12
4e12
0
Bound
0
2,000
4,000
6,000

FIGURE 3

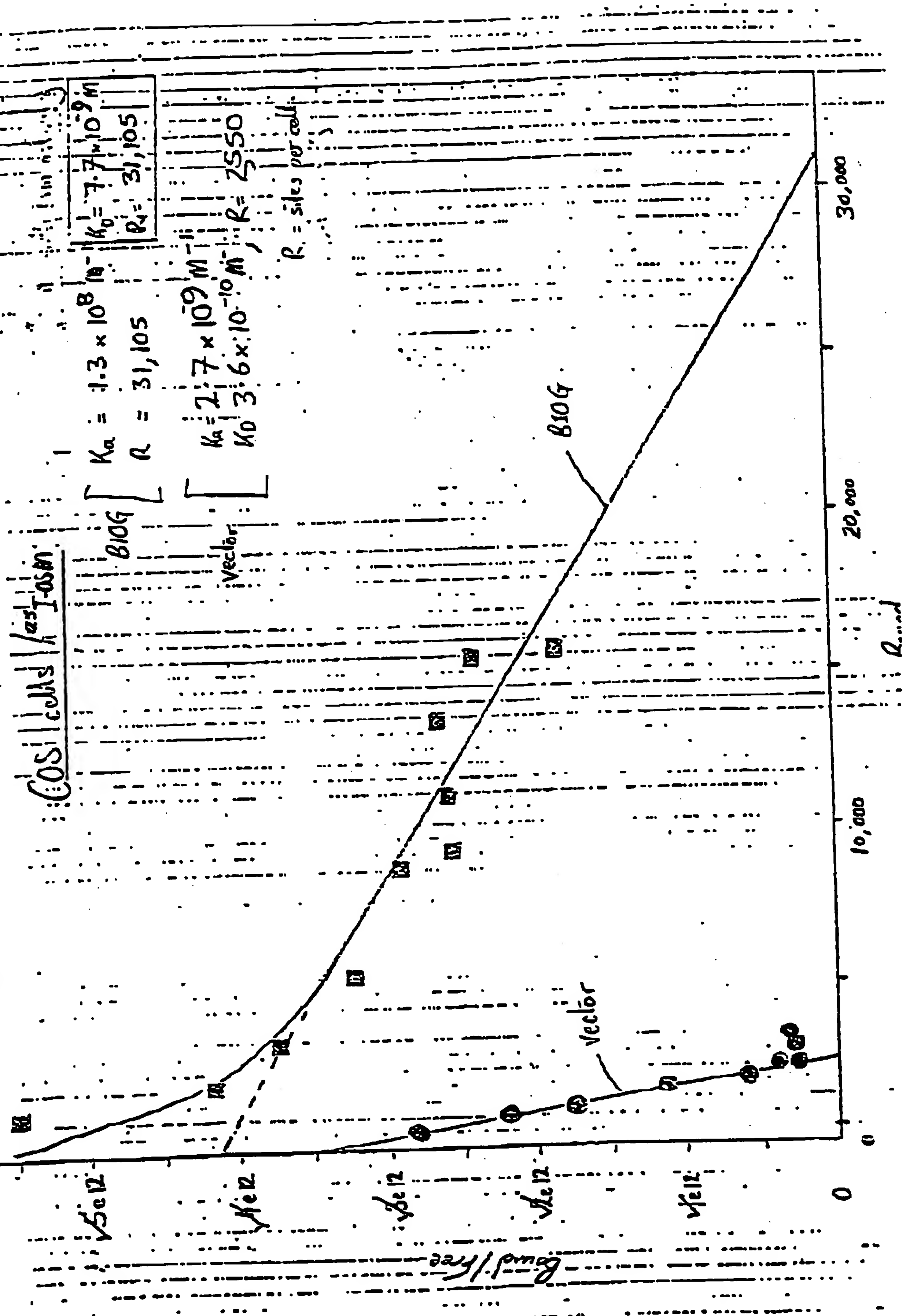
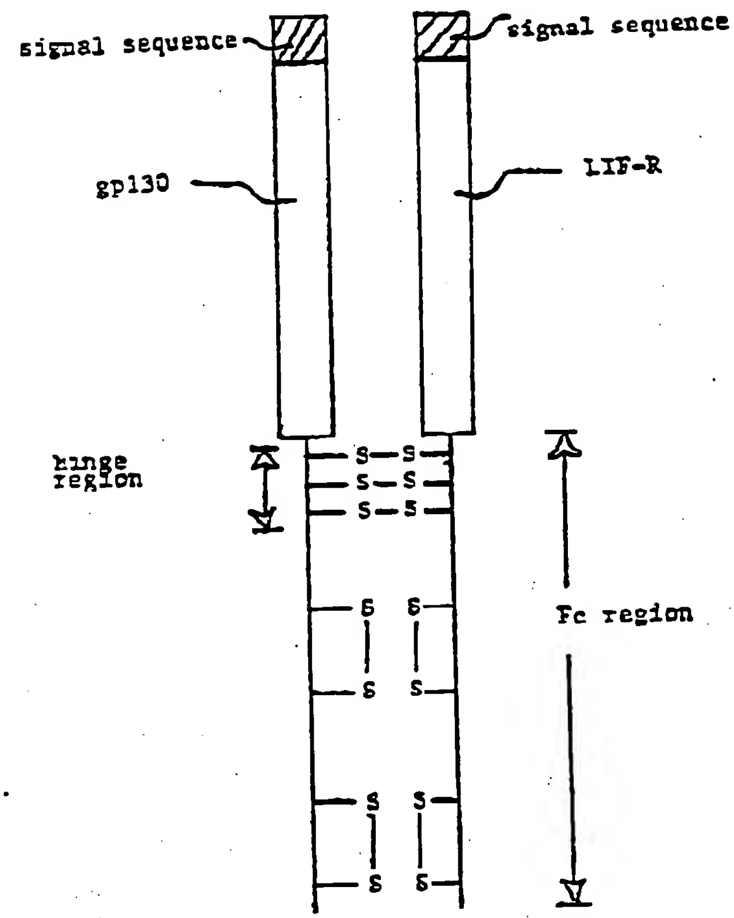


FIGURE 4


signal sequence
signal sequence
gp130
LIF-R
hinge region
S—S
S—S
S—S
S
S
S
S
S
S
S
S
Fc region

5/7

FIGURE 5

FIGURE 6

[illegible]

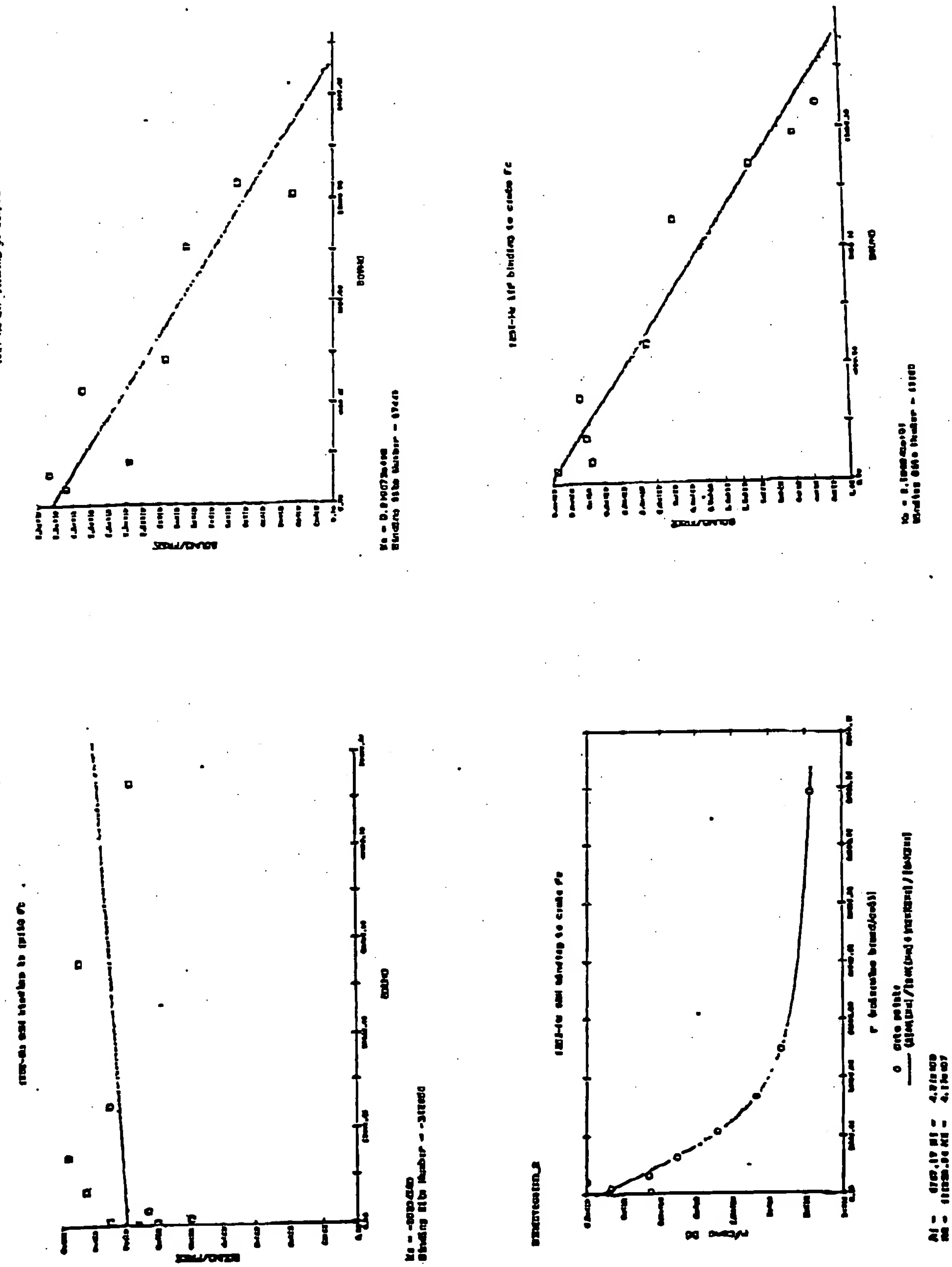


FIGURE 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10272

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07K 13/00; C12N 15/62
US CL :435/69.7; 514/2; 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 69.7, 252.3, 320.1; 514/2; 530/350; 536/27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN/MEDLINE
search terms: gp130, leukemia inhibitory factor, receptors

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EMBO Journal, Vol. 10, No. 10, issued October 1991, D. P. Gearing et al, "Leukemia inhibitory factor receptor is structurally related to the IL-6 signal trasducer, gp130", pages 2839-2848, see entire document.	1-20
A	Cell, Vol. 63, issued 21 December 1990, M. Hibi, et al, "Molecular Cloning and Expression of an IL-6 Signal Transducer, gp130", pages 1149-1157, see entire document.	1-20
A,P	US, A, 5,155,027 (Sledziewski et al) 13 October 1992, column 5, line 38 to column 6, line 16.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 March 1993	10 MAR 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer JOHN D. ULM Telephone No. (703) 308-0196